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Office des recherches sur les pêcheries du Canada
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Journal of the Fisheries Research Board of Canada

Trimethylamine-Producing Bacteria on Haddock (*Melanogrammus aeglefinus*) Fillets during Refrigerated Storage

R. A. Laycock and L. W. Regier

Fisheries Research Board of Canada
Halifax Laboratory, Halifax, N.S.

Laycock, R. A., and L. W. Regier. 1971. Trimethylamine-producing bacteria on haddock (*Melanogrammus aeglefinus*) fillets during refrigerated storage. J. Fish. Res. Bd. Canada 28: 305–309.

All of the psychrophilic groups of organisms isolated from haddock (*Melanogrammus aeglefinus*) fillets during storage at 3 C included organisms capable of producing trimethylamine (TMA) from trimethylamine oxide (TMAO). The percentage of TMA-producing isolates in the flora remained nearly constant during storage to spoilage, although the composition altered markedly. All *Pseudomonas putrefaciens* isolates were TMA producers and this organism was the most numerous TMA producer during storage. It was concluded that in this investigation *P. putrefaciens* was largely, if not wholely, responsible for observed TMA production. Achromobacters, although initially forming over half the TMA-producing flora, formed a decreasing percentage during storage and were not considered to contribute significantly to observed TMA production. Coryneforms were the only group to show an increase in percentage of TMA producers during storage.

Received August 17, 1970

The spoilage of fillets of lean fish during refrigerated storage is largely due to the metabolic activities of psychrophilic bacteria, in particular pseudomonads. The accumulation of the bacterial metabolite trimethylamine (TMA) during storage, together with its contribution to spoilage odours, has led to the widespread use of TMA production as an index of quality. The relation between TMA production and bacterial spoilage, however, is not clear-cut. It has been found (Tarr 1939; Shaw and Shewan 1968) that the characteristic odours of spoilage can occur independently of TMA production, depending on the nature of the bacterial inoculum. It is also possible that production of TMA could occur independently of spoilage, since TMA accumulates in fish muscle as an odourless salt and is converted to the "fishy" smelling base only in the later stages of spoilage.

A study was therefore made of the numbers and kinds of organisms capable of producing TMA that occur during spoilage of haddock (*Melanogrammus aeglefinus*) fillets packaged in oxygen-permeable film and stored at 3 C. The possible relation between TMA production and bacterial spoilage is discussed.

Printed in Canada (J1889)

Materials and Methods

Haddock fillets were obtained from a local processor and were all from trawler-caught fish graded No. 1 by inspectors of the Department of Fisheries and Forestry. The fillets were individually heat-sealed without vacuum in low density, 1.5-mil, polyethylene bags, and stored at 3 C.

Total bacterial counts, identification, and classification of organisms were made as described previously (Laycock and Regier 1970). Isolates termed achromobacters all belonged to the oxidase-positive *Moraxella*-like group of these organisms.

The methods of sensory evaluation of spoilage have also been previously described (Laycock and Regier 1970).

TMA nitrogen was determined by the method of Dyer (1945) on the same samples used in the bacteriological analyses.

The TMA-producing ability of all isolates was tested using a modification of the method of Wood and Baird (1943), which allowed simple rapid detection of TMA production during routine characterization of isolates.

Racks of tubes (Fig. 1) containing 2.5 ml of Wood and Baird's medium were inoculated along with other differential media using a multineedle inoculator. All isolates grew well in the medium and were tested for TMA production after 4 days at 20 C. Although TMA production on the fillets would be occurring at 3 C, the more convenient higher temperature of incubation of Wood and Baird's medium was considered justified. Pivnick (MS 1949) found that almost all cultures of examined fish bacteria capable of growing and producing TMA at 25 C could also do so at 3 C.

To test for TMA production, 0.75 ml of 40% formaldehyde was first added to each of the 20 tubes in a rack to combine with any ammonia present. The medium was then made alkaline by addition of 1.5 ml of saturated potassium carbonate to each tube. Cornwall pipettors were used for the addition of reagents. Besides the obvious advantage of speed of operation, the pipetting device by its action effected mixing of reagents and also made it relatively easy to avoid contamination of the mouths of the tubes with reagent. Immediately after the addition of the carbonate, the rack of tubes was covered with a petri dish lid containing a 9-cm filter paper, which had been soaked in 0.05% bromothymol blue, adjusted to *p*H 3 with 0.1 N sulphuric acid. The rack was then placed in a 45-C water bath. The presence of TMA was detected by a change in the indicator colour to blue (alkaline) at the mouth of a tube. Development of colour was usually very rapid, taking less than 30 sec, and the maximum incubation period found to be necessary was 2 min. During this time the next rack of tubes was prepared for testing. The testing of 300 isolates, including preparation of reagents, took less than an hour.

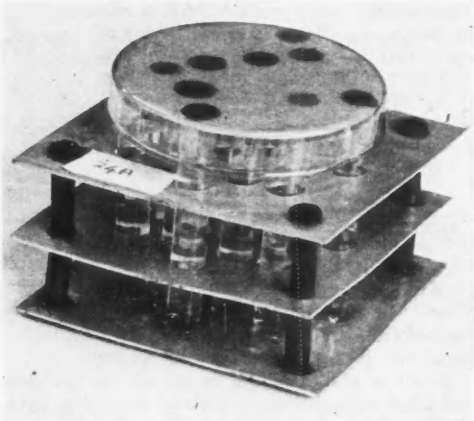

FIG. 1. Rack used in the detection of TMA-producing organisms. Dark areas at the mouths of tubes indicate that TMA is present.

Previous experiments using serial dilutions of TMA in lieu of trimethylamine oxide (TMAO) in sterile medium had shown the reaction to be about 10 times more rapid in the present method than in Wood and Baird's method. When this is taken into account the observed reaction time of 2 min in the present method is in fairly good agreement with the 30-min reaction time noted by Wood and Baird.

Since diffusion of TMA across the indicator paper results in an obscuring of the mouths of neighbouring tubes within 5 min, initial testing of the method included removal of all negative tubes for further incubation. None of the more than 200 isolates tested showed any sign of TMA production after a further 2-hr incubation at 45 C.

Our experiments thus indicated that with organisms growing well in the medium, TMA was either produced in large amounts or not at all. The minimum amount detectable in the 2-min reaction period was 200 μg, comparable with the 148 μg detected by Wood and Baird after 30 min. It was considered very unlikely that any nonspecific reactions in the relatively simple medium of Wood and Baird would interfere with the results. However, substrate blanks were performed on 100 TMA-producing organisms selected from each of the different groups of bacteria isolated. All were found to be negative (i.e., no change in indicator) after 1 hr of incubation.

Results

Organisms isolated from haddock fillets during storage at 3 C were: achromobacters, *Pseudomonas* groups I, II, and III/IV, *P. putrefaciens*, flavobacteria, vibrios, *Bacillus* spp., coryneforms, and micrococci. With the exception of *Bacillus* spp., nearly all of which were *B. subtilis*, all groups of isolates included organisms capable of producing TMA from TMAO.

The percentage of TMA producers in the flora (Fig. 2) remained nearly constant at

around 50 during storage before spoilage (8 days). Further storage resulted in a slight decline to 43% TMA producers after 13 days. All *P. putrefaciens* isolates throughout storage were TMA producers, whereas *Pseudomonas* I and II contained only a small percentage of TMA producers. Initially the percentage of TMA-producing achromobacters was over 60 but this declined during storage to 32% after 13 days. Coryneforms were the only group to show an increase in percentage of TMA producers during storage. Trimethylamine production was found to be associated with a group of coryneforms exhibiting a faint red fluorescence under ultraviolet light when grown on the medium of King et al. (1954). Initially the red fluorescing group constituted about half of the coryneform isolates but during storage nearly all coryneform isolates were found to be red fluorescent and an increasing percentage of these isolates were TMA producers. Micrococci were isolated most commonly immediately after filleting, about 40% of the isolates at this stage being TMA producers. During further storage micrococci were irregularly isolated and none of these subsequent isolates were found to be capable of TMA production. *Pseudomonas* III/IV and flavobacteria were only isolated in the early stages of storage and only in small numbers.

Total numbers of TMA producers (Fig. 3) increased during storage from 10^5/g to over 10^7/g at spoilage (8 days) but showed little further increase at 13 days. The most rapid increase in numbers of TMA producers before spoilage was shown by *P. putrefaciens*, which increased from 10^4/g to 10^7/g at spoilage and accounted then for 80% of all TMA-producing isolates. Storage up to 13 days resulted in little further increase in the numbers of this organism. Trimethylamine-producing *Pseudomonas* I and II, although increasing rapidly up to the time of spoilage, were present in much lower numbers than *P. putrefaciens*. The most numerous TMA-producing group after *P. putrefaciens* were the achromobacters, which increased steadily in numbers throughout the storage period and reached 10^7/g after 13 days. The rate of increase of TMA-producing achromobacters during storage before spoilage was, however, slower than that of *P. putrefaciens*, and while achromobacters initially formed 68% of all TMA-producing isolates they formed only 14% at spoilage. Postspoilage storage showed TMA-producing achromobacters forming an increased percentage of the TMA-producing flora as the rate of increase of *P. putrefaciens* declined. Coryneforms also increased rapidly during storage before spoilage but never formed more than a small percentage of the TMA-producing flora.

Discussion

Pseudomonads, the organisms mainly responsible for spoilage of fresh fish at low temperatures, have also been implicated in TMA production.

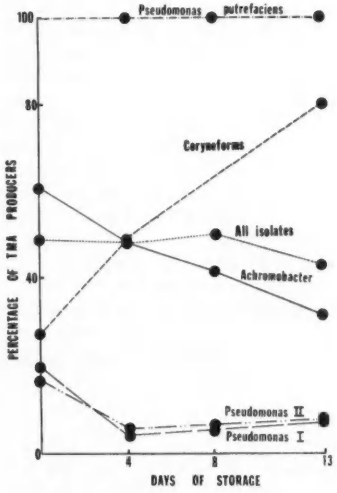

FIG. 2. Percentages of TMA producers in the different bacterial groups during storage at 3 C.

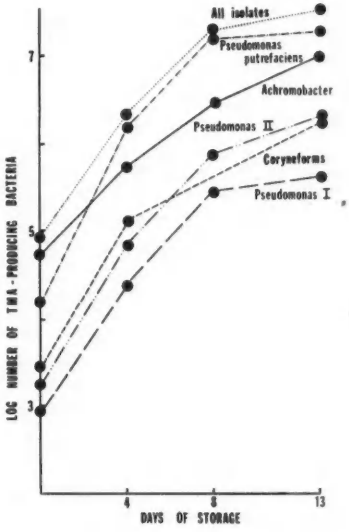

FIG. 3. Numbers of TMA-producing bacteria in the various groups during storage at 3 C.

It has been found (Power et al. 1964; Miyauchi et al. 1966; Pelroy and Seman 1969) that preservatives such as EDTA (ethylenediaminetetraacetic acid) and gamma irradiation, which selectively inhibit the growth or reduce the numbers of pseudomonads on fish, also depress TMA production. However, whereas organisms capable of actively spoiling fish can be found in all of the *Pseudomonas* groups (Castell et al. 1949; Chai et al. 1968; Shaw and Shewan 1968), the results of our experiments indicated that *P. putrefaciens* was predominant in TMA production, considerably outnumbering all other TMA-producing pseudomonads. A direct relation between the numbers of *P. putrefaciens* and TMA nitrogen, throughout storage, is suggested by Fig. 4.

Achromobacters, the only other group of TMA-producing isolates to form more than a very small percentage of the flora, were not considered to contribute significantly to total TMA production. This is suggested by the direct relation observed (Fig. 4) between the number of *P. putrefaciens* and TMA nitrogen even in the initial stages of storage, when achromobacters outnumbered *P. putrefaciens* by more than three to one. Further evidence of the relative inactivity of TMA-producing achromobacters was obtained from haddock fillets given 100 krad gamma irradiation. On the irradiated fillets achromobacters were the predominant group of isolates capable of TMA production, increasing logarithmically throughout the storage period (Fig. 5). However, there was a considerable lag in TMA production from the fillets and the eventual logarithmic increase in TMA production that occurred was found to coincide with the reappearance of significant numbers of pseudomonads in the flora.

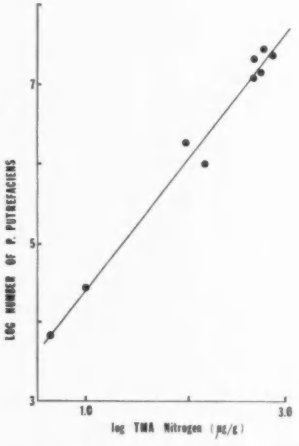

FIG. 4. Relation between numbers of *Pseudomonas putrefaciens* and total TMA production in fillets.

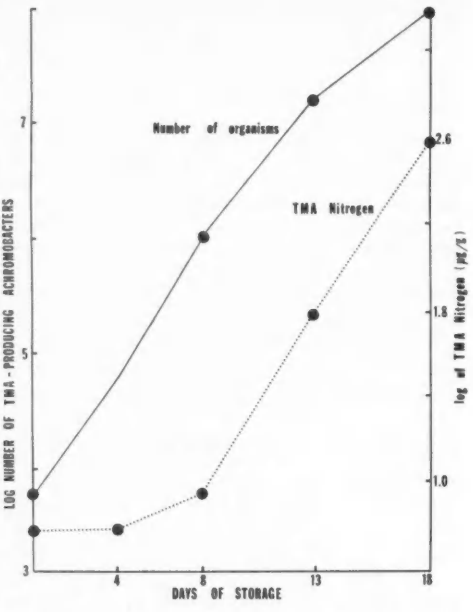

FIG. 5. Numbers of TMA-producing achromobacters and total TMA production in irradiated (100 krad) fillets during storage at 3 C.

Pseudomonas putrefaciens, as a result of the evidence presented, can be considered to be the source of TMA production in these experiments. Since this organism is also a particularly active spoilage organism of fish during refrigerated storage (Castell et al. 1969; Chai et al. 1968) an important link is established between bacterial spoilage and TMA production. Treatment with compounds such as EDTA, which has been found to selectively inhibit *P. putrefaciens*, leaving other spoilage pseudomonads unaffected (unpublished data), destroys this link and allows spoilage to occur while TMA production remains low.

Thus the usefulness of TMA production as an index of quality is dependent on the presence of *P. putrefaciens*, or organisms performing a similar role, in the spoilage flora.

Acknowledgments

The authors thank Mr C. H. Castell for many helpful suggestions, and Mr A. Longard for his assistance in the presentation of the data. This investigation was supported in part by Atomic Energy of Canada Limited.

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Crustacean Plankton of 146 Alpine and Subalpine Lakes and Ponds in Western Canada[1]

R. Stewart Anderson

Canadian Wildlife Service
University of Calgary Campus, Calgary, Alta.

Anderson, R. S. 1971. Crustacean plankton of 146 alpine and subalpine lakes and ponds in western Canada. J. Fish. Res. Bd. Canada 28: 311–321.

Investigations of crustacean plankton communities in 146 alpine and subalpine waters in some western Canadian national parks and environs showed that species composition varied little from year to year and that there was a mean of 2.93 species per community. *Diaptomus arcticus* occurred in 60% of the communities. More cladoceran species than copepod species were found, but there were usually more copepod than cladoceran species in a community. Many of the species found in subalpine waters did not occur in alpine waters, where they were probably limited more by physical factors — such as temperature and morphometry — and the presence of large predators than by altitude. Except for anostracans, few species seemed to be restricted to either lakes or ponds in the study area. Congeneric occurrences of large cladocerans or nonpredaceous diaptomid copepods were uncommon, as were co-occurrences of large cladocerans and nonpredaceous diaptomids.

Anderson, R. S. 1971. Crustacean plankton of 146 alpine and subalpine lakes and ponds in western Canada. J. Fish. Res. Bd. Canada 28: 311–321.

Des recherches sur les crustacés planctoniques de 146 lacs et étangs des zones alpines et subalpines ont été effectuées de 1965 à 1969 dans les parcs nationaux de l'Ouest du Canada. De façon générale, la composition des communautés est restée constante au cours des ans. Chaque communauté comprenait, en moyenne, 2.93 espèces de crustacés. Dans l'ensemble des lacs et étangs étudiés, il y avait plus d'espèces de cladocéres que d'espèces de copépodes. *Diaptomus arcticus* était l'espèce la plus fréquemment rencontrée. Les anostracés mis à part, seules quelques espèces étaient limitées soit aux lacs, soit aux étangs. Les associations congénériques de grands cladocéres ou de diaptomides non-prédateurs étaient rares, tout comme l'étaient les associations entre ces deux groupes.

Received September 18, 1970

The crustacean plankton species in alpine and subalpine lakes and ponds in various mountainous regions of the world are few and different. In general, single lake or pond communities contain only a few of the species that occur in a region (Löffler 1968a, 1969; Lötmarker 1964; Patalas 1964; Pennak 1957; Reed and Olive 1958; Stout 1969; Thomasson 1956; Valkanov 1938). Knowledge of zooplankton communities and species distribution in the numerous lakes and large ponds in the Canadian Rockies (over 1200 in the national parks of these mountains) is incomplete.

For the past 6 years, I have investigated the crustacean plankton communities of many lakes and ponds in all altitudinal belts in and near the Rocky Mountain national parks of southern Alberta and British Columbia. This paper pertains to the distributions of crustacean plankton species and the species compositions of plankton communities in alpine and subalpine waters in the study area. References to the distributions of some species in other altitudinal belts are also included.

The Study Area

Of the 146 lakes and ponds studied (Fig. 1, Table 1), most are in Banff, Jasper, Yoho, and Waterton Lakes national parks; a few are in forest reserves in Alberta and British Columbia. The waters were chemically similar: most contained between 50 and 150 ppm of total dissolved solids and, in all except a few dilute waters, calcium was the predominant cation and bicarbonate the predominant anion. Five lakes and ponds had salinities between 2 and 3 ppm, and these may represent some of the lowest salinities recorded for natural

[1]Carried out as part of the Canadian Wildlife Service program of limnological research in the Canadian National Parks.

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waters (cf. Livingstone 1963). Some details of the five most dilute waters are given in Table 2. Locations and water analyses for other representative waters of this study have been summarized elsewhere (Anderson MS 1968, 1970a, b).

Most of the isolated high-altitude waters are cirque lakes or tarns, a few are barrier lakes formed by rock slides, and some of the closely grouped waters, such as those near the headwaters of the Maligne and Brazeau rivers in Jasper National Park, are ice-scour lakes and ponds.

All 146 waters of this study are in the alpine or the subalpine belt. The subalpine belt is an altitudinal zone between the treeless alpine belt above and the approximate upper limit of the closed forest of the montane belt below (Löve 1970). The lower limit of the alpine belt is between 2200 and 2400 m in the study area, and that of the subalpine belt between 1550 and 1750 m.

Because the terms "lake" and "pond" defy precise definition (Macan and Worthington 1962; Reid 1961), the waters were arbitrarily categorized according to the graph in Fig. 2. Five astatic waters, exceeding pond dimensions at high water but not in August and September, were grouped with the ponds. These five and some other ponds probably dry up or freeze solid every year (indicated by abundance of anostracans). A few shallow lakes and ponds contain water throughout the year and are "perennial ponds" in the sense of Steinböck (1938). Except for a very few lower subalpine lakes and ponds, the waters studied supported no benthic macrophytes. For this reason, littoral and limnetic communities were not distinguished and all were considered limnetic (cf. Pennak 1957).

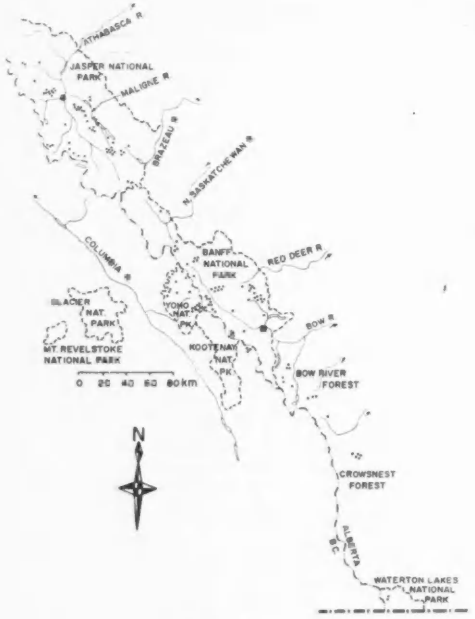

FIG. 1. Study area showing the distribution of the 146 lakes and ponds.

Methods

Most sampling was done in August, September, and October. Sampling of 25 of the waters in May, June, July, or during the winter, has produced no additional species. Most specimens (except cyclopoids) collected in winter or spring were immature, and therefore difficult or impossible to identify. Forty-two of the waters were sampled in more than one year, many three or more times, and one alpine lake more than 70 times over a 5-year period.

Plankton samples were collected with a Wisconsin-style plankton net (aperture size 76 μ; mouth diameter 25 cm), from a canoe or rubber dinghy, at the deepest part of the lake or pond. Each set of samples consisted of two or more vertical hauls from bottom to surface, plus two hauls from middepth in waters less than 10 m deep and two hauls from a depth of 5 m and two from 5 m above bottom in deeper ones. Occasionally samples were collected by throwing a weighted net from shore when ponds were very small and shallow, or when storms or mishaps precluded use of a dinghy; for most of these waters, vertical hauls were made from a boat in previous or subsequent years. Shore sampling missed daphnids in one lake, although the differential vertical sampling indicated that they were near bottom at the deepest part of the lake. In other waters, the same species were collected by the two methods, though the relative abun-

TABLE 1. Distribution of the lakes and ponds of the study area by altitudinal zone and by national park or forest reserve.[a]

	Alpine		Subalpine	
	Lakes	Ponds	Lakes	Ponds
Jasper	37	10	6	4
Banff[a]	21(23)	9	16(18)	3
Yoho	10	3	4	3
Waterton Lakes	–	–	1	1
Alta. forest reserves	2	2	6	5
B.C. forest reserves	1	–	1	1
Total	71(73)	24	34(36)	17

[a]Numerals in parentheses include four waters considered twice because plankton composition was known before and after fish stocking.

TABLE 2. Locations, water chemistry, and number of zooplankton species for five dilute lakes in Jasper National Park, Alta.

Lake	Collection date	Elev. (*m*)	Grid location	Specif. conduct. (*μmhos at 25 C*)	Sum of constituents (*ppm*)	Ca	Mg	HCO_3	SO_4	SiO_2	No. species[a]	
											Copepods	Cladocerans
South Cairngorm[b]	12-IX-68	2430	183650MJ	5.0	2.4	0.1	0.1	0.4	1.1	0.50	2	0
Beacon[b]	28-VIII-69	2145	140210MJ	6.6	2.7	0.8	0.2	1.4	0.2	0.01	1	1
Chalk[c]	28-VIII-69	2210	152195MJ	4.9	–	–	–	–	–	–	1	0
Pond[c]	28-VIII-69	2320	378140MJ	5.2	–	–	–	–	–	–	1	2
Lick[c]	28-VIII-69	1967	404103MJ	3.1	–	–	–	–	–	–	1	1

[a]All species present were among the most frequently occurring (Fig. 3).
[b]Laboratory and field analyses.
[c]Field analyses only.

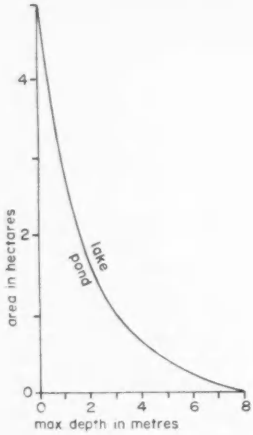

FIG. 2. Curve used for designation of waters as lakes or ponds.

dance of species sometimes differed. Pirocchi (1949) and Patalas (1964) have discussed the deficiencies of sampling from shore.

In four of the 146 waters, introduction of fish during the study resulted in a marked change in the zooplankton species present, and each of the four waters was considered as having two distinct communities.

Methods for physical and chemical measurements have been summarized elsewhere (Anderson 1970a). Except for *Ceriodaphnia affinis*, identification of and terminology for crustacean plankton species follow the keys of Dexter, Brooks, Wilson, and Yeatman (Edmondson 1959).

Annotated List of Species Found

ANOSTRACA

Branchinecta paludosa (O.F. Müller) 1788. In alpine and high subalpine ponds with no higher aquatic vegetation; ponds often turbid; usually near bottom in deep, astatic waters; pigmented individuals in clear, shallow ponds; reproductive peak usually in late August and September, males persisting up to 2 months longer than females.

Eubranchipus intricatus Hartland-Rowe 1967. In two subalpine ponds with some higher aquatic vegetation; ponds often turbid; reproductive peak in July.

Streptocephalus seali Ryder 1879. In one subalpine pond with *E. intricatus*; individuals often green with epiphytes; reproductive peak in July.

Artemiopsis stephanssoni Johansen 1922. In one turbid, alpine ice-scour pond (cf. Hartland-Rowe and Anderson 1968); reproductive peak in late August and September.

COPEPODA

Diaptomus shoshone S. A. Forbes 1893. Abundant in astatic subalpine waters; less abundant in alpine lakes and ponds; usually in early thawing waters and with one or more congeneric species; reproductive peak near freezeup.

Diaptomus arcticus Marsh 1920. The most common plankton species in this study and the only one present in many waters; frequently abundant at the surface; less common in subalpine ponds than in subalpine lakes or alpine waters; reproductive peak near freezeup in most waters of this study.

A common spring-pond species on the prairies (Anderson 1970b; Hammer and Sawchyn 1968).

Diaptomus leptopus S. A. Forbes 1882. In one shallow subalpine lake; reproduction throughout the year.

More common in shallow montane lakes and prairie ponds in Alberta (Anderson 1970b, MS 1970b; Reed MS 1959).

Diaptomus arapahoensis Dodds 1915. In alpine and subalpine lakes and perennial ponds south of Banff National Park; reproductive peak near freezeup.

Diaptomus franciscanus Lilljeborg 1889. In one subalpine perennial pond south of Banff National Park.

Diaptomus victoriaensis Reed 1958. Very abundant in one astatic alpine pond; pond frequently turbid; reproductive peak in August and September (cf. Anderson 1968).

Diaptomus tyrrelli Poppe 1888. Common in alpine lakes and subalpine ponds; in alpine lakes, one generation per year and reproductive peak near freezeup; in subalpine ponds, often two generations per year with reproduction beginning in July.

Diaptomus sicilis S. A. Forbes 1882. In four subalpine lakes; not usually abundant.

More common in montane lakes, where reproductive peak usually occurs during period of ice cover (Anderson 1970c; Reed MS 1959); found in prairie waters with salinities of 5000 ppm or more (Anderson 1970d).

Diaptomus nudus Marsh 1904. In one alpine pond; sometimes present one year and not the next; probably only one generation per year.

More common in montane and prairie ponds, where two or more generations are produced per year (Anderson 1970b, MS 1970a, MS 1970b).

Diaptomus ashlandi Marsh 1893. In one cold, turbid, subalpine lake; *Cyclops vernalis* the only other crustacean plankter present; reproduction in September.

Cyclops bicuspidatus thomasi S. A. Forbes 1882. Most common in lakes; often abundant when *D. arcticus* or *D. shoshone* absent; usually one generation per year with reproductive peak in spring or early summer; in some lakes, a second generation may produce an additional smaller peak in autumn.

Cyclops vernalis Fischer 1853. The most common cyclopoid in the waters studied, though often sparsely represented; most abundant during winter in some of the lakes (cf. Anderson MS 1968); reproductive peak usually in early winter in lakes, but during July or August in some alpine ponds.

Because of wide variations in life cycle, morphology, and habitat, *C. vernalis* may constitute a complex of sibling species (Price 1958; Reed MS 1959; Z. Brandl personal communication).

Macrocyclops albidus (Jurine) 1820. In three subalpine lakes; usually sparsely represented.

More common in montane lakes in the study area (Anderson 1970c; Reed MS 1959).

Macrocyclops ater (Herrick) 1882. In one subalpine perennial pond; infrequent (cf. Carl 1940).

Orthocyclops modestus (Herrick) 1883. In two deep, cold, subalpine lakes, near bottom.

More common in some small montane lakes in the general study area (e.g., Herbert Lake; Anderson 1970a).

Eucyclops agilis (Koch) 1838. Sparse in subalpine lakes and ponds.

Common in montane lakes with extensive littoral zones (Anderson 1970c, MS 1970b; Reed MS 1959).

CLADOCERA

Daphnia pulex Leydig 1860 emend. Richard 1896. Usually abundant where found, sometimes in very turbid waters; frequently near bottom in deep lakes; less common than *Daphnia middendorffiana* in alpine lakes.

More common in many montane and prairie waters (Anderson 1970c, MS 1970b; Anderson and Fabris 1970).

Daphnia middendorffiana Fischer 1851. Often numerous in alpine and upper subalpine lakes and alpine ponds; pigmentation greater with diminishing lake depth and turbidity; frequently abundant at surface; peak ephippia production usually in late July.

Daphnia schødleri Sars 1862. In two subalpine lakes.

Daphnia rosea Sars 1862 emend. Richard 1896. In two subalpine lakes; usually abundant only when congeneric species absent.

Daphnia galeata Sars 1862 *mendotae* Birge 1918. In three subalpine lakes; usually abundant only when congeneric species absent.

Holopedium gibberum Zaddach 1855. In one clear alpine lake, one turbid alpine pond, and one clear subalpine pond; abundant where found.

Scapholeberis kingi Sars 1903. In subalpine lakes and ponds; not usually abundant.

More common in montane lakes with littoral zones (Anderson 1970c, MS 1970a).

Simocephalus vetulus Schoedler 1858. In subalpine lakes and ponds and in one alpine pond, usually with *Daphnia pulex*; sometimes absent one year and present the next (cf. Anderson 1968); peak ephippial production usually in July, about a month earlier than *D. pulex*.

Bosmina longirostris (O. F. Müller) 1785. In one alpine and two subalpine lakes; infrequent.

More common in montane lakes (Anderson 1970c, MS 1970b).

Alonella nana (Baird) 1850. In one subalpine perennial pond, where abundance varied markedly from year to year.

Abundant in some montane lakes with littoral zones (Anderson 1970c, MS 1970b).

Alona guttata Sars 1862. Infrequent in several alpine lakes and subalpine lakes and ponds.

Alona affinis (Leydig) 1860. Rare; in one subalpine perennial pond.

Alona rectangula Sars 1861. Rare; in one alpine and one subalpine lake, and one subalpine pond.

Alona costata Sars 1862. Fairly abundant in one alpine pond.

Chydorus sphaericus (O. F. Müller) 1785. The most frequently occurring cladoceran in the waters studied, but often sparsely represented in alpine waters.

Chydorus gibbus Lilljeborg 1880. Rare; in one subalpine lake and one perennial pond.

Ceriodaphnia affinis Lilljeborg 1900. Fairly abundant in one alpine pond, two subalpine lakes, and three ponds.

Identification based on general description being closest to those of *C. affinis* by Manuilova (1964) and Šrámek-Hušek et al. (1962) and teeth of proximal pecten of the postabdominal claw being distinctively smaller than the corresponding teeth in *C. reticulata* from montane lakes in the general study area (cf. Anderson MS 1970b).

Polyphemus pediculus (Linné) 1761. Fairly common in two subalpine lakes.

More common in small montane lakes with extensive littoral zones (Anderson 1970c, MS 1970a, MS 1970b).

Community Composition

By the end of 1967, 64 communities were studied. In the following 2 years, 82 more lakes and ponds were sampled and some of the original 64 reinvestigated. Although the total number of crustacean plankton species found increased from 21 to 38, the mean number of crustacean species per plankton community rose only from 2.83 to 2.93 species. Of the 38 species in the 146 waters, 11 occurred only once (Table 3). *Diaptomus arcticus*, *Cyclops vernalis*, and *Chydorus sphaericus* occurred most frequently, although the last two were few in number in some lakes and ponds. The three most common co-occurrences involved these same three species (Fig. 3).

Thirty of 42 lakes and ponds sampled in more than 1 year during late summer and early autumn showed no increase in the number of species present. One or two additional crustacean species were collected from each of 10 of the remaining 12 waters, but these species were sparsely represented, never more than about 1% of the total number of crustaceans present and often represented by no more than three or four individuals. In two ponds sampled in more than two years, *Simocephalus vetulus* occurred erratically, moderately abundant one year and absent the next. In one of these ponds, *Diaptomus nudus* was abundant the year *S. vetulus* was absent, and was absent the year *S. vetulus* was present. It is interesting that in the case of Snowflake Lake

(Anderson MS 1968, 1970a), sampled over 70 times, more species were collected in the first samples than in any subsequent samples. Here, too, the additional species were represented by no more than three or four individuals.

The mean number of planktonic crustacean species per community was 2.62 in alpine waters and 3.53 in subalpine waters (Table 4). All subalpine waters contained planktonic crustaceans, but six alpine waters had none (although rotifers or dipteran larvae were present). The distribution of numbers of species in the communities was sharply unimodal for alpine waters, but not for subalpine waters (Fig. 4). The numbers of species per community were more randomly distributed for ponds than for lakes in both altitudinal belts (Fig. 4). In both belts, the mean number of species occurring in ponds (Table 4) were not significantly different from those in lakes ($P = 0.1$).

The mean number of copepod species per water body (1.70) was higher than that of cladoceran species per water body (1.14). The number of cladoceran species was generally higher in ponds than in lakes, but the number of copepod species was generally highest in lakes. The range of numbers, of cladoceran species per water body was much greater for subalpine waters than for alpine waters; the range for copepod species per water body was the same, or less, for subalpine waters than for alpine waters. Anostracans were confined to astatic waters or small ponds in both altitudinal zones (Table 4).

TABLE 3. Frequency of occurrence of crustacean plankton species in lakes (L) and ponds (P) in the alpine (A) and subalpine (S) belts of the study area.[a]

Species	AL	AP	SL	SP
Anostraca				
Branchinecta paludosa	–	7	–	4
Eubranchipus intricatus	–	–	–	2
Streptocephalus seali	–	–	–	1
Artemiopsis stephanssoni	–	1	–	–
Copepoda				
Diaptomus shoshone	8	3	1	1
Diaptomus arcticus	59	11	19	1
Diaptomus leptopus	–	–	1	–
Diaptomus arapahoensis	2	1	3	1
Diaptomus franciscanus	–	–	–	1
Diaptomus victoriaensis	–	1	–	–
Diaptomus tyrrelli	17	2	4	10
Diaptomus sicilis	–	–	4	–
Diaptomus nudus	–	1	–	–
Diaptomus ashlandi	–	–	1	–
Cyclops bicuspidatus thomasi	13	3	9	4
Cyclops vernalis	21	10	18	7
Macrocyclops albidus	–	–	3	–
Macrocyclops ater	–	–	–	1
Orthocyclops modestus	–	–	2	–
Eucyclops agilis	1	0	3	2
Cladocera				
Daphnia pulex	8	5	5	7
Daphnia middendorffiana	20	3	7	2
Daphnia schödleri	–	–	2	–
Daphnia rosea	–	–	2	–
Daphnia galeata mendotae	–	–	3	–
Holopedium gibberum	1	1	–	1
Scapholeberis kingi	–	–	3	2
Simocephalus vetulus	–	1	3	2
Bosmina longirostris	1	–	2	–
Alonella nana	–	–	1	–
Alona guttata	5	–	2	2
Alona affinis	–	–	1	–
Alona rectangula	1	–	1	1
Alona costata	–	1	–	–
Chydorus sphaericus	29	13	14	8
Chydorus gibbus	–	–	1	1
Ceriodaphnia affinis	–	1	2	3
Polyphemus pediculus	–	–	2	–

[a]Numerals refer to the numbers of waters in which each species was found. Dash (–) indicates that the species was not found in that category.

Interspecific Association

The coefficients of interspecific association (C_7; Cole 1949) were calculated for each of the nine most frequently occurring species or groups of species in the 146 waters (Fig. 5). In the calculations, when twice the standard error (2 SE) was numerically less than the calculated C_7, a statistically significant difference from randomness ($C_7 = 0$) occurred. As C_7 approached +1 it approached perfect positive association, and as C_7 approached –1 it approached perfect negative association.

Four of seven significant positive associations involved anostracans. These four indicate the type of high-altitude pond community to be expected with anostracans. It is notable that the C_7's for *Diaptomus arcticus* (the most common crustacean species in the 146 waters) were only highly significant in two instances — positive with *Daphnia middendorffiana* and negative with *Daphnia pulex*. As expected, the negative C_7 for these two *Daphnia* species was highly significant. It is also notable that *D. middendorffiana* was positively associated with one of the predaceous diaptomids (*Diaptomus arcticus*) and that *D. pulex* was positively associated with the other (*Diaptomus shoshone*). Significant negative C_7's were less frequent than significant positive C_7's, but the three that occurred were highly significant. Four of five C_7's for congeneric occurrences were negative and two of these were highly significant.

Of the four most common predaceous copepod species in the study (Fig. 3), *D. arcticus* and *Cyclops bicuspidatus thomasi* were usually very abundant where they occurred, whereas *D. shoshone* and *C. vernalis* were often present in only small numbers and *C. vernalis* was found to reach peak abundance during winter or early spring in many lakes. Although the last two species may feed at rates higher than the first two (Anderson 1970b), the effects of predation on interspecific association are undoubtedly reduced by low predator abundance and by seasonal succession. This view is supported by the fact that most C_7's for *D. arcticus* and *C. b. thomasi* were negative, whereas only two of eight C_7's

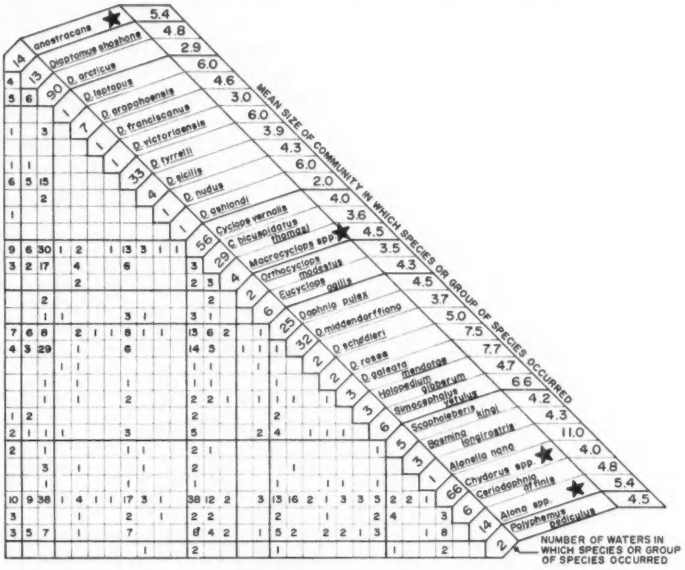

FIG. 3. Co-occurrence chart for crustacean plankton species in 150 communities. Four waters were considered twice because plankton composition was known before and after fish stocking. Numerals in the body of the chart are the numbers of times two species occurred together (e.g., *Diaptomus arcticus* occurred 29 times with *Daphnia middendorffiana*). Note grouping of anostracans and three other genera (all except anostracans and 2 *Alona* spp. were single occurrences, often low in abundance).

TABLE 4. Numbers of species in three groups of crustacean plankton in the various types of water.[a]

		Anostracans			Copepods			Cladocerans			Total crustaceans		
Type of water	No. waters	Mean no.	Range	Total	Mean no.	Range	Total	Mean no.	Range	Total	Mean no.	Range	Total
Alpine lakes	73	0	0	0	1.70	0–4	7	0.88	0–4	7	2.57	0–6	14
Alpine ponds	24	0.33	0–1	2	1.33	0–3	7	1.08	0–3	6	2.75	0–6	15
Subalpine lakes	36	0	0	0	1.94	1–4	12	1.42	1–8	6	3.33	1–11	18
Subalpine ponds	17	0.41	0–2	3	1.70	1–4	9	1.76	0–4	9	3.94	1–8	21
All alpine waters	97	0.08	0–1	2	1.61	0–4	8	0.93	0–4	8	2.62	0–6	18
All subalpine waters	53	0.12	0–2	3	1.87	1–4	13	1.53	0–8	17	3.53	1–11	33
All waters	150	0.10	0–2	4	1.70	0–4	16	1.14	0–8	18	2.93	0–11	38

[a]Four waters were considered twice because plankton composition was known before and after fish stocking.

were negative for each of the other two species (Fig. 5).

Distribution of Species

Except for species that occurred only once or twice in the waters studied, only the anostracans (mainly *Branchinecta paludosa*) were restricted to ponds and only *Diaptomus sicilis*, *Macrocyclops albidus*, and *Bosmina longirostris* to lakes (Table 3). Some species most common in alpine lakes (*Diaptomus arcticus*, *D. tyrrelli*) and some occurring only in subalpine lakes (*Daphnia schødleri*, *D. rosea*, *D. galeata mendotae*, *Polyphemus pediculus*, *Diaptomus sicilis*, *D. leptopus*, *Orthocyclops modestus*, *M. albidus*) are common pond forms at lower altitudes (Anderson MS 1970a, MS 1970b; Anderson and Fabris 1970; Carl 1940; Cole 1953). Except for the anostracans and possibly *Diaptomus victoriaensis*, none of the species of this study appear to be strictly permanent- or temporary-water forms in the study area and its environs.

This study and others (Anderson 1968, MS 1968, 1970a, b, c, d, MS 1970a, MS 1970b; Bajkov, 1929; Rawson MS 1940; Reed MS 1959) conducted in the general study area (Fig. 1) indicate that only *Diaptomus shoshone* and *Daphnia middendorffiana* were restricted to the subalpine and alpine belts. *Branchinecta paludosa*, *Daphnia pulex*, *Chydorus sphaericus*, *Simocephalus vetulus*, *Scapholeberis kingi*, *Diaptomus tyrrelli*, *Cyclops vernalis*, and *C. b. thomasi* were fairly to very common in all altitudinal belts. *Diaptomus arapahoensis*, *Eucyclops agilis*, *Ceriodaphnia affinis*, *Daphnia schødleri*, *D. rosea*, and *D. g. mendotae* were common in the subalpine and lower belts. *Eubranchipus intricatus*, *Streptocephalus seali*, *Diaptomus nudus*, *D. leptopus*, *D. sicilis*, *Macrocyclops albidus*, *Orthocyclops modestus*, *Polyphemus pediculus*, *Bosmina longirostris*, *Alonella nana*, and *Alona* spp. were rare or infrequent in the alpine and subalpine belts, though more frequent at lower altitudes. *Diaptomus arcticus*, common on the prairies as well as in the alpine and subalpine belts, was seldom found in the intermediate altitudinal belts. *Diaptomus victoriaensis*, *D. franciscanus*, *D. ashlandi*, *Macrocyclops ater*, and *Holopedium gibberum* occurred too infrequently in the general study area to justify generalizations on altitudinal distribution.

Only two species showed patterns of regional distribution: *D. arapahoensis* was found only in waters south of Banff National Park; *D. shoshone* occurred in the same region and in south-central Jasper National Park; *H. gibberum* and *Scapholeberis kingi* were abundant in widely separated permanent and temporary waters. *Artemiopsis stephanssoni* and *D. victoriaensis* may represent isolated populations 1500–2000 km south of the next nearest reported occurrences. Regional and sporadic patterns in the distribution of crustacean plankton species, and the marked differences between communities in adjacent waters, indicate the need to gather plankton samples and basic limnological data from as many waters in a region as possible.

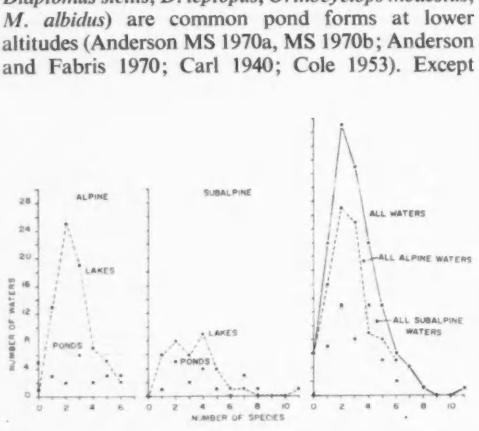

FIG. 4. Distributions of numbers of crustacean species per community in the various types of waters.

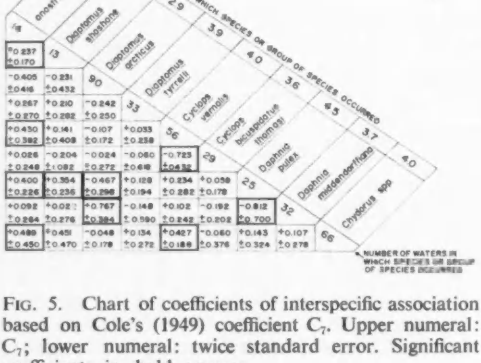

FIG. 5. Chart of coefficients of interspecific association based on Cole's (1949) coefficient C_7. Upper numeral: C_7; lower numeral: twice standard error. Significant coefficients in bold squares.

General Discussion

On the basis of species distribution, this study provides little evidence for ecologically definitive limits between lakes and perennial ponds in the study area. Pond species in one region or altitudinal belt were found in lakes in another, and vice versa. However, the presence or absence of anostracans and the generally larger mean number of crustacean species in anostracan communities (5.4 species)

than in all communities studied (2.93 species) are indications of fundamental ecological differences between intermittent and permanent waters.

There are justifiable objections to categorizing certain crustacean species as "planktonic" in the strictest sense, even when they are collected in the limnetic zone. Hutchinson (1967) suggests the terms "limnoplankton" for lakes and "heleoplankton" for ponds but, as already observed, the distinction between lakes and ponds is unclear. I have collected the usually planktonic species *D. arcticus* only near bottom or littorally in certain mountain lakes containing fish, and have found that some species not usually considered planktonic (e.g., *C. vernalis*) occur persistently or frequently in the limnetic zones of certain lakes. Similarly, some littoral species have occurred limnetically in lakes after fish and many crustacean competitors were eradicated with rotenone (e.g., *Acroperus harpae* and *Scapholeberis kingi*; Anderson 1970c). In line with Hutchinson's (1967) terminology, some species might be more precisely described as belonging to the meroplankton (e.g., *C. vernalis*, *Macrocyclops albidus*) or to the pseudoplankton (e.g., *Eucyclops agilis*, *M. ater*). However, the proliferation of descriptive terminology will contribute little to a better understanding of the taxonomic status, ecology, or life cycle of many poorly known aquatic crustacean species.

Undue emphasis on the importance of a rigorous environment as a factor limiting the number of species and their total abundance is indeed possible (Green 1969). However, the low species diversity in the plankton of the alpine and most of the subalpine lakes likely results from uniform and somewhat inhospitable physical environments that provide few ecological niches. In some of the lakes, low temperatures, very low primary production, and a short growing season combine to make conditions barely adequate for the existence of certain species that mature as diminutive adults with very low reproductive rates (Anderson MS 1968). Suboptimal conditions for growth and reproduction, rather than altitude per se, undoubtedly impose an upper altitudinal limit on the distribution of certain species.

Cyclopoid copepods were often sparse in many of the high-altitude lakes and ponds, perhaps partly because of diaptomid predation (Anderson 1970b) and partly because cyclopoids seem to be less adaptable than diaptomids to the physical environment of these waters. *Orthocyclops modestus*, for example, requires comparative cold to complete its life cycle (Ewers 1936), but most of these lakes are probably too cold for satisfactory growth and reproduction. Both cyclopoids and diaptomids can eat a variety of foods (Anderson 1970b). However, when temperatures are very low and food is scarce, individuals of many cyclopoid species take longer to complete each instar and eventually reach an adult size nearly constant for each species (Coker 1933; Ewers 1936; Smyly 1961). In contrast, adults of some diaptomid species, such as *D. shoshone*, *D. arcticus*, and *D. tyrrelli* (Anderson MS 1968, 1970b), have shown more than a two-fold difference in body length (Fig. 6), the smallest occurring where conditions were most rigorous. Length variations of this magnitude represent up to a 10-fold difference in body volumes. Diaptomids are apparently more suitably adapted than cyclopoids to the rather short season of tolerable conditions characteristic of most high-altitude lakes of this study.

The occurrence and abundance of some species may vary according to the response of various species to threshold values for certain physical and chemical characteristics. Species distribution is probably related both to mean temperatures and to large fluctuations in diurnal temperatures (cf. Löffler 1968a). Species such as *Simocephalus vetuhus*, *Eubranchipus intricatus*, and *Diaptomus nudus*, which occurred erratically from year to year in high ponds, are more common in warmer waters at lower altitudes. Although temperatures in some of the alpine and subalpine lakes may seldom or never exceed 8 C, these lakes may exceed 4 C and be ice-free for a longer period each year than nearby lakes because of exposure, the amount of melt water received, and other factors. A longer period of tolerable conditions may be the factor that permits such species as *Diaptomus shoshone*, *D. arapahoensis*, *D. leptopus*, *D. ashlandi*, *D. sicilis*, and *Alonella nana* to occur in certain high altitude lakes and not in others.

Some notable features of the co-occurrence chart (Fig. 3) are the absence of congeneric occurrences of nonpredaceous diaptomid copepods (species other than *D. arcticus* and *D. shoshone*), the infrequency of co-occurrences of large cladocerans, and the few co-occurrences of large cladocerans with nonpredaceous diaptomids. Supported by the C_7's for the more abundant species or groups of species, these distributional features indicate similarity in the niche requirements of nonpredaceous diaptomids and large daphnids.

Significant association coefficients (C_7's) are useful indicators of the "typical" crustacean plankton communities of the high-altitude waters. In the study area and its environs, an alpine or subalpine lake will most likely contain *Diaptomus arcticus* and *Daphnia middendorffiana* and possibly one other crustacean species. In certain regions of the study area, lakes may contain *Daphnia pulex*,

Diaptomus shoshone, and possibly two other crustacean species or, in the absence of predaceous diaptomids, *Cyclops vernalis*, *Chydorus sphaericus*, and possibly two other species. The typical fairy shrimp pond will probably contain one anostracan (usually *Branchinecta paludosa*), *Diaptomus shoshone*, *D. pulex*, *C. vernalis*, and *C. sphaericus*.

The limnetic communities of many mountain lakes have remained comparatively stable over a period of many years (Baldi 1951; D'Ancona 1955), although sudden or gradual changes in the composition of the zooplankton of alpine lakes do occur (Pirocchi 1948; Pennak 1955). In this study, there was little evidence for large changes in the composition of the crustacean plankton, except in four previously fish-free lakes where stocked fish (*Salmo gairdneri* or *Salvelinus fontinalis*) eliminated larger crustacean species, and in a few ponds where two or three crustacean species fluctuated greatly in abundance from year to year. A "stabilizing selection" (Dillon 1970) is undoubtedly exerted on the zooplankton in lakes by the short period of tolerable physical conditions and by competition and predation. Greater instability and more species may occur in some communities because competition is lower when conditions are variable and inconsistent from year to year (Cole 1963, 1966; Anderson and Fabris 1970). Unstable conditions in two of the small alpine lakes was suggested by the co-occurrence of three species of diaptomids and one cyclopoid, three of the four species being predators.

Although passive dispersal of aquatic invertebrates by mammals has been demonstrated (Maguire 1963), regional differences in climate and exposure, rather than differences in passive dispersal mechanisms, are probably responsible for the regional occurrence of certain species in lakes of the alpine or subalpine belts. Birds are important vectors in the dispersal of aquatic crustaceans (Proctor 1964; Löffler 1968b). The northern phalarope (*Lobipes lobatus*), possibly the most important dispersal vector in the lakes of this study, migrates southward through the study area in autumn when the diaptomid copepods and anostracans are carrying egg clutches. It has been observed on some of the highest and most isolated of the lakes studied where it may eat as many as 120 copepods per minute. It is unlikely that many waters in the area escape potential colonization by the most commonly occurring zooplankters.

Acknowledgments

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(FIG. 6 *opposite*)

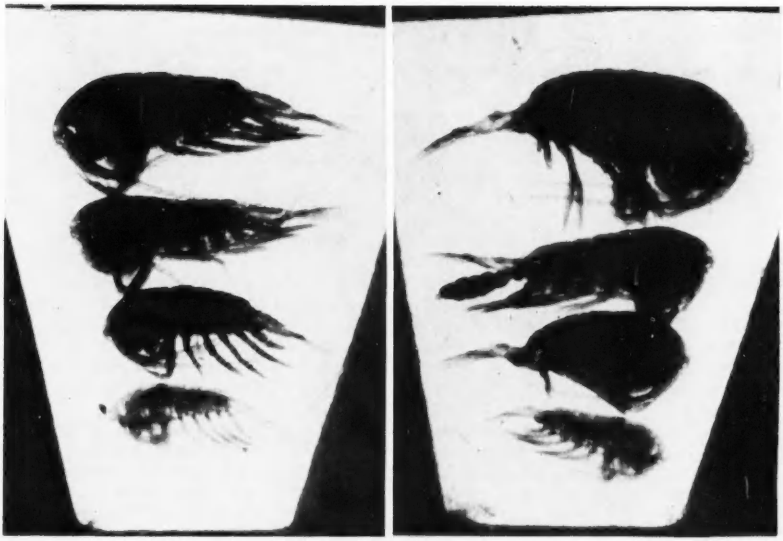

FIG. 6. Adult *Diaptomus shoshone* from Plateau and Teardrop ponds (Anderson 1968) and from Nigel (863886MI) and Lazuline (829158MJ) lakes in Jasper National Park (males, left; females, right).

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Species of *Lepeophtheirus* (Copepoda, Caligidae) Recorded from the Ocean Sunfish (*Mola mola*) and their Implications for the Caligid Genus *Dentigryps*

G. C. HEWITT[1]

Fisheries Research Board of Canada
Biological Station, Nanaimo, B.C.

HEWITT, G. C. 1971. Species of *Lepeophtheirus* (Copepoda, Caligidae) recorded from the ocean sunfish (*Mola mola*) and their implications for the caligid genus *Dentigryps*. J. Fish. Res. Bd. Canada 28: 323–334.

Of the five species of *Lepeophtheirus* so far recorded from the ocean sunfish (*Mola mola*), *L. ornatus* Edwards has been previously synonymized with *L. nordmanni* Edwards. A comparison of specimens shows that *L. insignis* Wilson is also a synonym of *L. nordmanni* and that *L. molae* Heegaard is a synonym of *L. hastatus* Shiino. *Lepeophtheirus nordmanni* and *L. hastatus* are redescribed and compared. *Lepeophtheirus hastatus* can be distinguished from *L. nordmanni* by an accessory branch on its postantennary spine; in the female *L. hastatus*, the fifth legs are styliform whereas in female *L. nordmanni* they are lamelliform; the male of the latter species has a reduced sternal furca whereas male *L. hastatus* have a sternal furca extending beyond the interpodal bar.

The great similarity between the two species casts doubts on most previous records of *Lepeophtheirus* from *M. mola* and the known distribution of these parasites is discussed.

Since the fifth legs of *L. hastatus* are very similar to those found in *Dentigryps* Wilson, and these legs are the only distinguishing feature of this genus, the species previously placed in *Dentigryps* are transferred to *Lepeophtheirus*. The following new combinations are proposed: *Lepeophtheirus curtus* (Wilson, 1913) n. comb., *L. litus* (Lewis, 1964) n. comb., *L. ulua* (Lewis, 1964) n.comb., and *L. longicauda* (Cressey, 1966) n.comb. *Lepeophtheirus lewisi* nom.nov. is proposed for *D. bifurcatus* Lewis, 1964.

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Lepeophtheirus nordmanni (Edwards 1840)

Caligus nordmanni Edwards 1840 p. 455.
Caligus ornatus Edwards 1840 p. 455.
Lepeophtheirus nordmanni (Edwards) Baird 1850 p. 275; Heller 1868 p. 180; Scott 1900 p. 151; Scott and Scott 1912–13, p. 66; Wilson 1905 p. 623; 1932 p. 411; 1935 p. 778; Shiino 1957 p. 359; 1959 p. 347; 1960a p. 487; Stuardo 1958 p. 1; Threlfall 1967 p. 171.
Lepeophtheirus insignis Wilson 1908 p. 444; Hewitt 1964 p. 153.

MATERIAL EXAMINED

(All specimens from *Mola mola*, the ocean sunfish.) 4 females, 4 males — north of Rona Bay, Eastbourne, New Zealand, collected by R. A. Falla, November 30, 1960. Dominion Museum, Wellington, Collection. 1 male — Mana Is., New Zealand, collected by R. Fordham, April 3, 1964, taken in a large collection of *L. hastatus*. To be deposited with the Dominion Museum, Wellington. 1 adult female, 1 juvenile female — 58°00′N, 0°20′E, collected by L. Fauskanger, October 14, 1933, identified as *L. nordmanni* by Stuardo (1958) by comparison with type material (Zool. Mus. Bergen No. 45017). 8 females — 120 miles South of Wood's Hole, Mass., August 1, 1900. Identified as *L. nordmanni* by C. B. Wilson. (U.S. Nat. Mus. Cat. No. US74304). 4 females, 4 males — coast of California. Cotypes and paratypes of *L. insignis* (U.S. Nat. Mus. Cat. No. US-74370). 2 females, 1 male — Kuki, Japan, December 25, 1962. Identified as *L. nordmanni* by S. M. Shiino. 10 females — Avalon Peninsula, Nfld., no date. Identified as *L. nordmanni* by W. Threlfall.

DESCRIPTION

Female (*Fig. 1–15*)

Overall length 11.3–12.6 mm (11.8).[2]

Cephalothorax subovate, a little longer than wide, 5.4–6.9 mm (6.0) × 5.0–6.2 mm (5.8). Frontal area half as wide as carapace 2.3–2.8 mm (2.6), rather short, usually with a slight medial invagination, but

[1]On leave from: Zoology Department, Victoria University of Wellington, Wellington, New Zealand.

[2]Measurements are given in the form: minimum–maximum (average). Measurements are given from the New Zealand material only; however, all the specimens examined were measured and do not differ markedly from the New Zealand specimens.

Printed in Canada (J1901)

sometimes extended anteriorly in midline. Lateral margin of cephalothorax and anterior margin of frontal plate with well-developed flanges, H-shaped dorsal grooves well developed, and extended medially from the anterior tips to meet the eyes in a further transverse line, with further less distinct grooves running anterolaterally from the eyes and laterally from the lateral margins of the more anterior transverse groove. Posterior sinuses well developed, the narrow strip of posterior median area, which borders them laterally, raised into a distinct rib that appears spinous in dorsal view. Posterior angles of this section extending slightly into the sinus. Outer margin of sinus bearing a semicircular flange. Free thoracic segment much wider than long, 0.9–1.1 mm (1.0) × 1.7–1.9 mm (1.8).

Genital segment longer than wide, 3.4–3.7 mm (3.6) × 3.1–3.5 mm (3.3), lateral margin rounded, forming an entire curve with the lateral portion of the posterior margin that forms broadly rounded posterior processes. Median part of posterior margin, at point of attachment of abdomen, sublinear. The segment somewhat depressed on either side of the midline in preserved material.

Abdomen longer than wide, 1.3–1.7 mm (1.5) × 1.2–1.3 mm (1.2), lateral margins curved, narrowing anteriorly and posteriorly. In some specimens there is a sudden narrowing just posterior to anterior margin, which suggests the presence of indistinct segmentation. Lateral margins somewhat flattened in preserved material.

Anal laminae longer than wide, 0.34–0.40 mm (0.37) × 0.24–0.30 mm (0.27), subrectangular, the posterior angles slightly rounded, with three long pilose setae on posterior margin, a further pinnate seta one-third this length on outer distal angle, two short setae, one on inner distal angle and one just medial to seta on outer distal angle, and a short row of setules along distal quarter of inner margin of lamina.

Egg strings not present in the New Zealand material, but in other material from 7 to 12 mm in length with 200–500 eggs per string.

First antenna (Fig. 4) two-segmented. First segment twice length of second, length twice width, outer margin bent near midpoint, with a spinous structure proximal to the bend, 12 pilose setae on outer margin distal to bend, a further 9 longer pilose setae on or near rounded outer distal angle (plumose nature of setae not shown in Fig. 4), and a further 2 small setae near midline of dorsal surface. Second segment width two-fifths length, with 13 setae on distal margin, 4 of these the result of incomplete bifurcation of 2 setae, a further seta just distal to midpoint of inner margin.

Second antenna (Fig. 5 and 6) with a subtriangular basal segment that has its outer posterior angle expanded as a blunt spine. Second part two-fifths length of third, width three-quarters length. Third part an elongate subchela, which is broadly curved, rounded near the base but with inner margin flattened near the sharp tip, basal width one-sixth length, a small spine on anterior margin near base, and a further spine on anterior margin one-third distance from base.

Postantennary spine (Fig. 5) one-third length of second segment of second antenna, basal section three times width of base of spine, spine slightly curved ending in a blunt tip.

First maxilla (Fig. 5) with three setae on a raised boss, the outermost twice length of inner two. The posterior spine bifid for distal half of its length, inner tine more slender than outer, the whole spine slender in appearance, nearly as long as postantennary spine.

Second maxilla (Fig. 7) of two segments. First segment two-thirds length of second, rounded distally. Second segment width one-tenth length, narrowing slightly distally, with a broad membranous flange just distal to midpoint, and two extensions. Subterminal extension two-thirds length of the other, with a narrow serrated membrane on its outer margin, the terminal extension with narrow membrane on both margins, proximal half of outer membrane serrated.

Maxilliped (Fig. 8) of two parts, subchelate. The first part two-fifths as long as wide, narrowing to half this width distally, subchela basal width one-fifth length, strongly curved near tip, ending in a sharp point, with a small spine two-fifths distance from proximal margin.

Sternal furca (Fig. 3) well developed and elongate, reaching posteriorly well beyond the interpodal plate of first pereiopods in most specimens. The tines are slender with basal width one-sixth length, half as long again as basal portion, diverging rapidly over proximal half of length, turning medially to become subparallel over distal half of length, middle third marked with a number of transverse narrow ridges, the tines bluntly rounded distally.

On either side of the sternal furca the medial terminations of transverse ribs of the cephalothorax have become swollen into spinelike structures, which are one-fifth length of furca.

First pereiopod (Fig. 9 and 14) exopod first segment a little longer than protopod and terminal exopod segment; protopod width two-thirds length, with a reduced endopod one-seventh length of exopod on inner distal angle. Endopod rounded distally without ornamentation, narrowed slightly near midpoint. Protopod with pinnate seta on outer distal angle and a further small pinnate seta near midpoint of inner margin. First exopod segment width two-thirds length, with a row of setules on inner margin and a small spine on outer distal angle. Second segment subrectangular, width half length, with three pinnate setae on inner margin, a small pinnate seta on inner distal angle, and three spines on distal margin. The outermost of the large spines has a small toothed area near its tip and a membrane with very fine serrations on its inner margin, second spine slightly smaller, with a finely serrated membrane on inner and outer margins, and an auxiliary spine one-third its length situated near the tip; third spine similar to second but smaller. Each spine with a membrane at its base.

Second pereiopod (Fig. 10) biramous, protopod as long as exopod, endopod a little shorter. Protopod with a small pinnate seta on outer distal angle, a large pinnate seta on inner proximal angle, and a membrane on inner margin; second exopod segment as long as third, one-quarter length of first. First exopod segment width half length, with a spine two-fifths segment length on outer distal angle and a further very small spine near its base, and a pinnate seta on inner margin near inner distal angle. Second exopod segment with a large spine on outer distal angle and a pinnate seta on inner distal

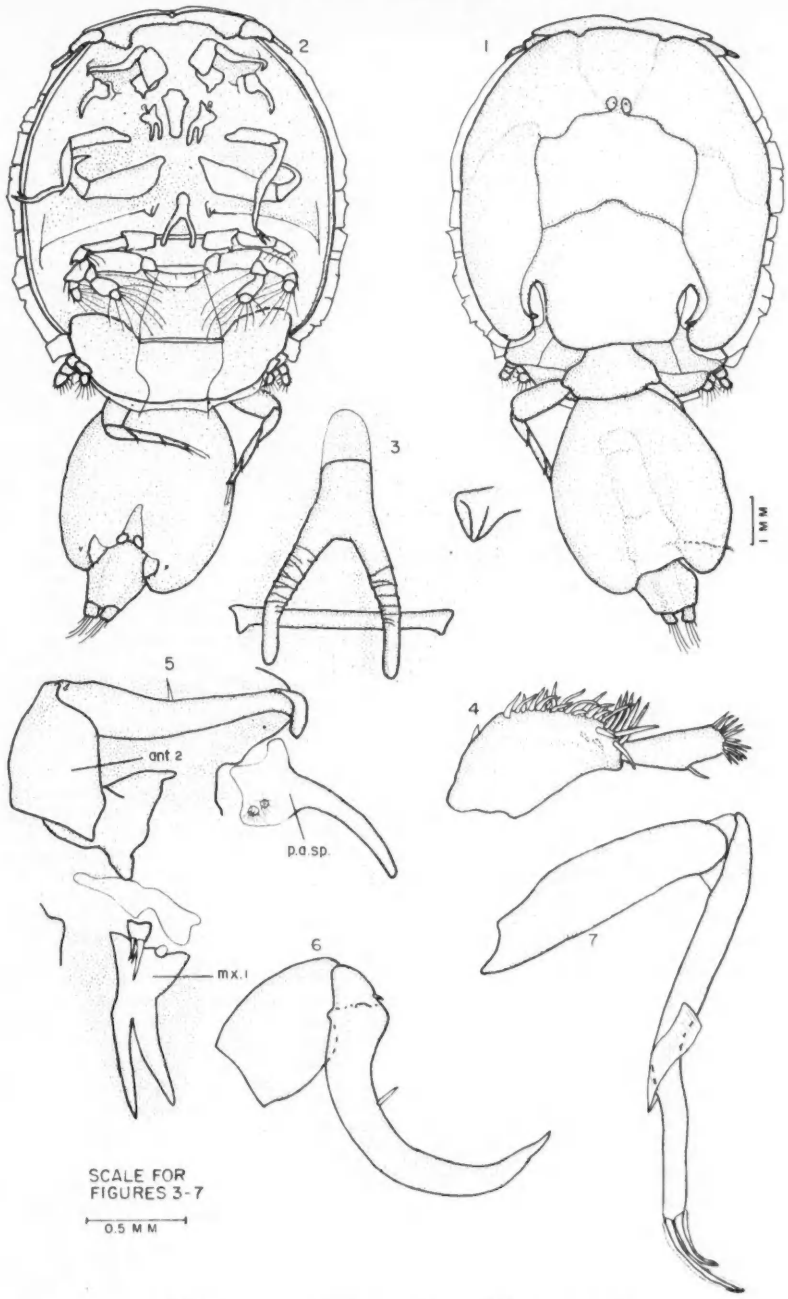

FIG. 1–7. *Lepeophtheirus nordmanni* (Edwards), female.
Fig. 1. Dorsal habitus; Fig. 2. Ventral habitus; Fig. 3. Sternal furca; Fig. 4. First antenna; Fig. 5. Second antenna (*ant. 2*), postantennary spine (*p.a.sp.*), and first maxilla (*mx. 1*); Fig. 6. Second antenna; Fig. 7. Second maxilla.

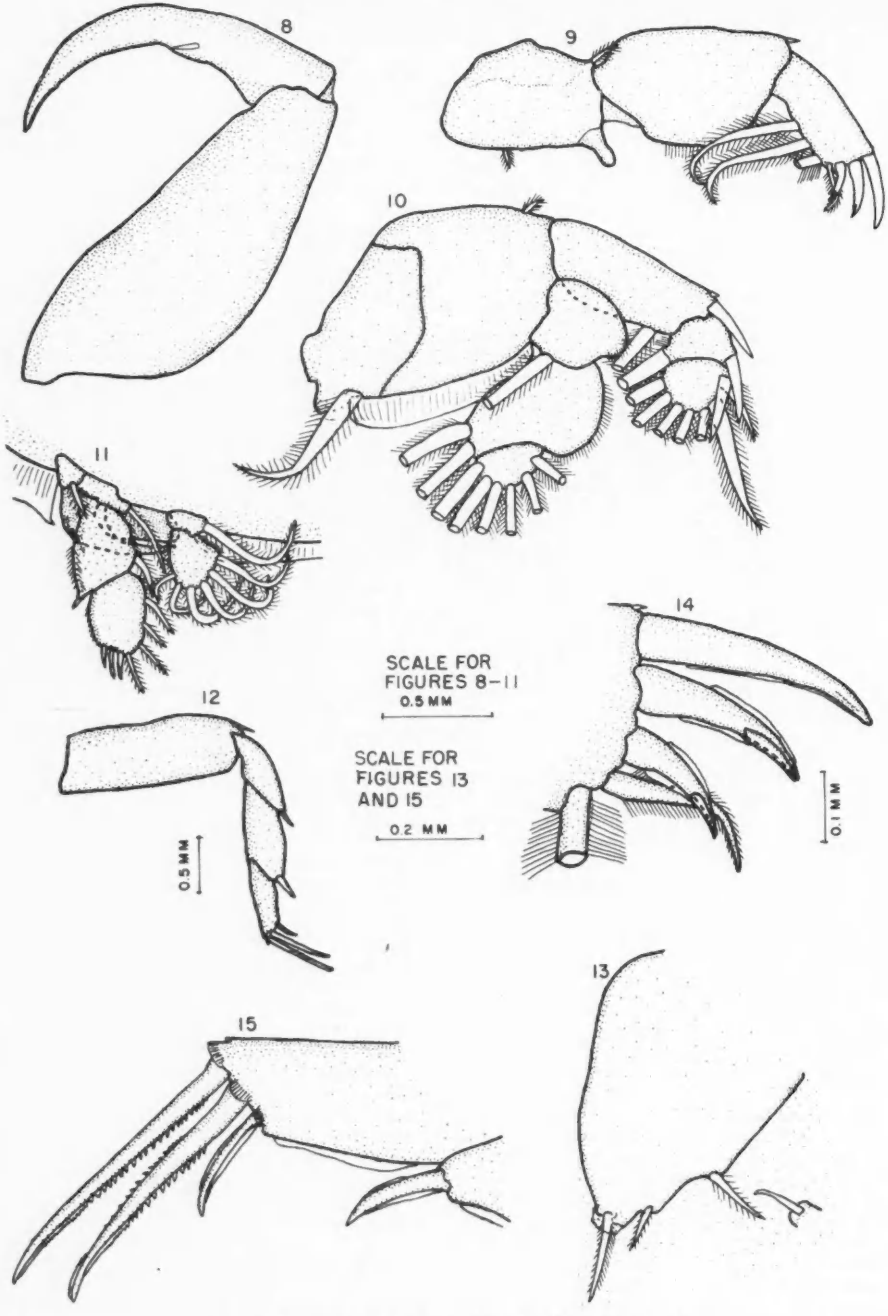

FIG. 8–15. *Lepeophtheirus nordmanni* (Edwards), female.
Fig. 8. Maxilliped; Fig. 9. First pereiopod; Fig. 10. Second pereiopod; Fig. 11. Third pereiopod; Fig. 12. Fourth pereiopod; Fig. 13. Fifth pereiopod; Fig. 14. First pereiopod, detail of distal extremity; Fig. 15. Fourth pereiopod, detail of distal extremity.

angle. Third segment with a large spine on outer margin, a pinnate seta of equal length beside it, and six longer pinnate setae on remainder of curved and united inner distal and outer margins. Third endopod segment half length of first, two-fifths length of second. First endopod segment with pinnate seta on inner margin, outer margin expanded, rounded and with a group of setae just distal to its midpoint. Second segment with outer margin rounded and bearing setules, inner distal angle expanded and bearing two pinnate setae. Third segment subsemicircular, proximal margin sublinear, with six pinnate setae on free margin.

Third pereiopod (Fig. 11) exopod twice length of endopod. First exopod segment very short with a pinnate seta on the inner and outer margins. Second segment as long as third, with setules on inner and outer margins, and small spine on outer distal angle and a pinnate seta on inner distal angle. Third exopod segment rounded distally, with three spines terminally, a row of setules on outer margin, and four pinnate setae on outer and outer-distal regions. The hook associated with exopod well developed, slightly overlapping endopod. Endopod of two segments, the first very short, bearing a pinnate seta on inner margin, the second rounded distally, with setules on inner and outer margins, and six pinnate setae on distal margin.

Fourth pereiopod (Fig. 12 and 15) of four segments, the distal three subequal in length, the protopod segment equal in length to the other three together. Protopod segment width one-third length, with a seta on outer distal angle. Second segment with outer margin longer than inner, outer margin bordered by a membrane that is finely serrated and tipped distally by a spine that has a finely serrated membrane on either side. Third segment similarly armed. Fourth segment with a similar membrane on inner margin, tipped by three spines on distal margin, the outermost half length of other two and with a finely serrated membrane on both margins, the middle spine with larger toothlike serrations on middle third of each margin, becoming a finely serrated membrane toward the tip, outermost margin with toothlike serrations merging into a finely serrated membrane on the outer margin only, all three terminal spines with pectinate membranes near their bases.

Fifth pereiopod (Fig. 13) flattened and leaflike, situated on ventral surface of genital segment near point of attachment of egg strings. Basal width three-quarters length, narrowing and somewhat rounded distally, the outer margin curving medially near the midpoint and toward the tip, a pinnate seta being placed at both these points, a further pinnate seta at the tip has its base surrounded by a short membrane with a finely serrated margin. Just lateral to the fifth pereiopod on the ventral surface of the genital segment is a raised area bearing a small seta.

Male (*Fig. 16–24*)

Overall length 6.9 mm.

Cephalothorax similar to that of the female, but proportionally a little wider (3.9 × 3.9 mm). Free thoracic segment similar in shape to that of the female, 0.6 × 1.0 mm.

Genital segment subcyclindrical, 1.2 × 1.5 mm, expanded laterally by the two dorsoventrally flattened semilobate rami of the fifth pereiopod.

Abdomen subrectangular, a little longer than wide, 0.8 × 0.7 mm, divided into two segments by a groove, one-quarter of distance from anterior margin.

Anal laminae (Fig. 24) as in female.

Appendages as in female except as described below.

Second antenna (Fig. 18) of two parts. Basal part a little longer than subchela, longer than wide, its surface marked by several patches of ridging, and with a cone-shaped swelling distally that also has a pattern of ridges. Second part basal width half length, branched one-third distance from tip, one branch one-third length of the other, both ending in sharp points, with a spine near base of segment, and a further spine near midpoint.

First maxilla (Fig. 20) with three setae on a raised boss, one seta half length of second, which is half length of innermost. Spine with basal width two-thirds length, has a very small branch one-third distance from tip, and a spine near tip that is one-quarter length of the whole spine.

Maxilliped (Fig. 21) as in female except for a subtriangular swelling on inner margin of first part one-third distance from distal margin.

Sternal furca (Fig. 22) very reduced, the tines only half length of basal portion, reaching only two-fifths distance from base toward interpodal plate of first pereiopod. However, spines on either side of it, developed from transverse rib, are proportionally longer than in female and themselves reach nearly to interpodal plate.

Fifth pereiopod (Fig. 23) consisting of two lobes subequal in length. The more anterior lobe with basal width two-thirds length, outer margin rounded, inner margin sublinear, with three pinnate setae on distal half of outer margin, and a further pinnate seta at the tip, which has a membrane around its base similar to that found associated with the terminal seta in the fifth pereiopod of the female. Posterior lobe, basal width subequal to length, with two small setae on distal half of outer margin, and a terminal seta with a flange similar to that of the anterior lobe.

Lepeophtheirus hastatus Shiino, 1960

Lepeophtheirus insignis Wilson; in Barnard 1948 p. 247; 1955 p. 251.

Lepeophtheirus hastatus Shiino 1960b p. 512.

Lepeophtheirus molae Heegaard 1962 p. 169; Hewitt 1964 p. 153.

Material Examined

38 females, 1 male, 1 juvenile female — Mana Is., New Zealand, collected by R. Fordham, April 3, 1964, (also contained 1 male *L. nordmanni*) to be deposited with the Dominion Museum, Wellington. 14 females (mounted on slides) — from Canterbury University, New Zealand, presumably from the Canterbury region. 1 female — Seto, Japan, March 25, 1953, paratype of

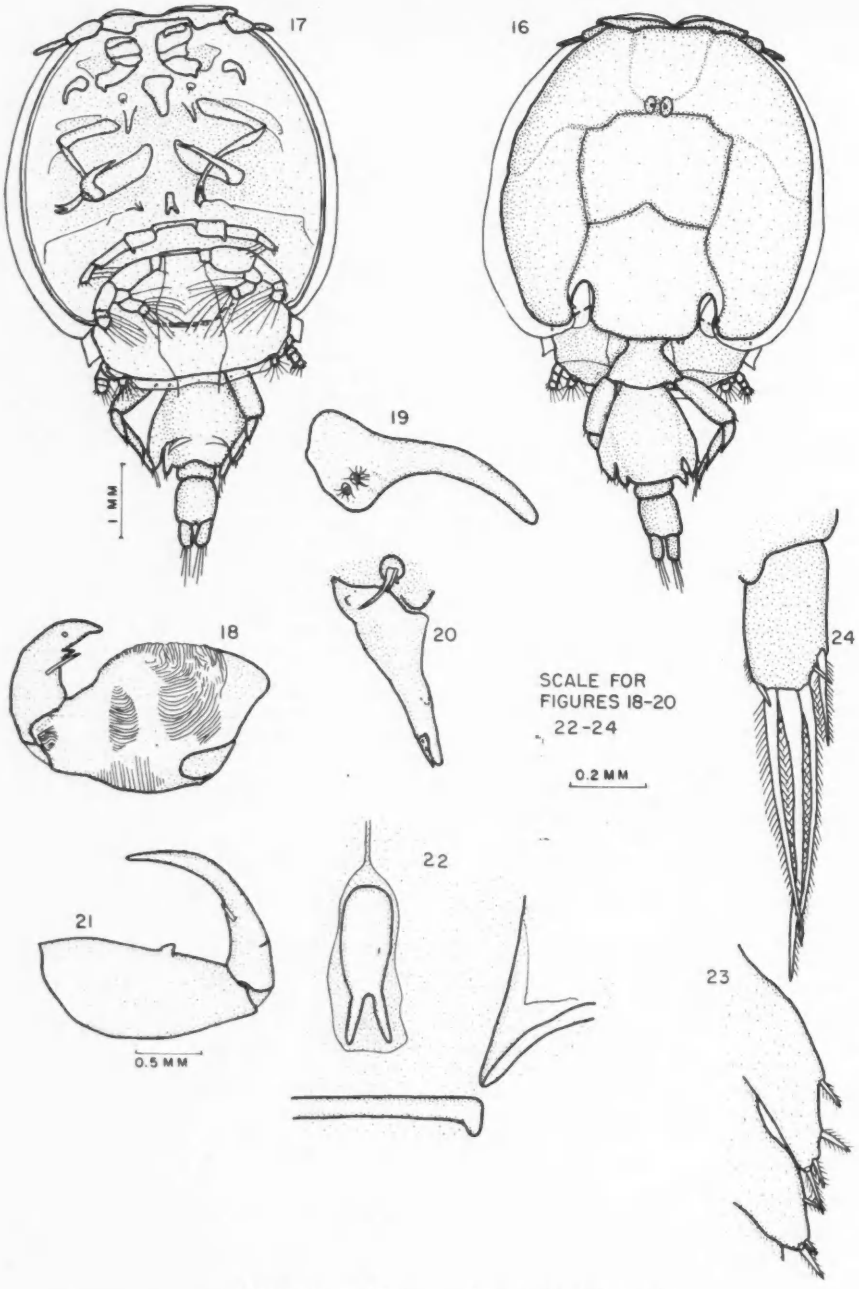

FIG. 16–24. *Lepeophtheirus nordmanni* (Edwards), male.
Fig. 16. Dorsal habitus; Fig. 17. Ventral habitus; Fig. 18. Second antenna; Fig. 19. Postantennary spine; Fig. 20. First maxilla; Fig. 21. Maxilliped; Fig. 22. Sternal furca; Fig. 23. Fifth pereiopod; Fig. 24. Anal lamina.

L. hastatus. 39 females, 1 juvenile female — Port Jackson, Sydney, December 13, 1882, paratypes of *L. molae* (Australian Mus. Reg. No. P16400 (part)). 10 females — Botany Bay, New South Wales, December 8, 1930. Paratypes of *L. molae*. (Australian Mus. Reg. No. P9990).

DESCRIPTION

Female (*Fig. 25–31*)

Overall length 10.2–12.3 mm (11.4).

Cephalothorax as in *L. nordmanni* but comparatively larger, 6.3–7.7 mm (6.9) × 6.9–8.2 mm (7.5). Free thoracic segment as in *L. nordmanni*, 0.7–1.1 mm (0.98) × 1.5–1.9 mm (1.69).

Genital segment a little wider than long, 2.0–2.9 mm (2.6) × 2.6–3.4 mm (3.0), the swollen posterior angles more broadly rounded than in *L. nordmanni* but somewhat variable in shape, some specimens with these angles extending more posteriorly than in the specimen shown in Fig. 25.

Abdomen two-segmented, overall dimensions 1.1–1.5 mm (1.21) × 0.72–0.91 mm (0.76). Both segments subrectangular, similar in width, the first segment one-third length of second.

Anal laminae similar in shape and armament to *L. nordmanni*, 0.21–0.38 mm (0.25) × 0.17–0.26 mm (0.17).

Appendages as in *L. nordmanni* except as below.

Postantennary spine (Fig. 27) with an additional small spine, one-quarter length of major spine, arising from the outer posterior angle of the base.

First maxilla with three unequally sized setae on a raised boss (Fig. 28), spine as in *L. nordmanni*.

Sternal furca (Fig. 29) has its branches continuing to diverge slightly beyond their midpoint, not parallel as in *L. nordmanni*, and the spines on either side of it developed from the transverse rib proportionally somewhat larger. As in *L. nordmanni* the furca normally reaches beyond the interpodal plate of first pereiopod but it does not always reach so far posteriorly.

Fourth pereiopod has on the outer distal angle of first segment in place of spine in *L. nordmanni* a raised spinous projection, with a lateral swelling that carries a plumose seta (Fig. 30).

Fifth pereiopod (Fig. 31) long and styliform, reaching posteriorly nearly to the end of the abdomen in some specimens. Basal width one-fifth length, narrowing gradually to a sharp point distally, with four sparsely pinnate setae the most proximal two-fifths distance from base, the most distal one-seventh distance from tip.

Male (*Fig. 32–36*)

Overall length 6.4 mm.

General body form as in male of *L. nordmanni*, except that the cephalothorax is a little larger and the genital segment a little narrower; cephalothorax 4.3 × 4.4 mm; fourth freely articulated thoracic segment 0.4 × 0.8 mm; genital segment 1.3 × 1.1 mm; abdomen overall measurement 1.0 × 0.55 mm; first abdominal segment half length of second; anal laminae 0.31 × 0.15 mm.

Appendages as in male of *L. nordmanni* except that the postantennary spine (Fig. 34) bears a small accessory spine as in the female of *L. hastatus*, which, however, is much smaller than in the female, only one-ninth length of major spine; first maxilla (Fig. 35) dividing into two subequal branches one-third distance from tip, one tine with an auxiliary spine as in *L. nordmanni*; sternal furca (Fig. 36) much better developed than in male *L. nordmanni*, overlapping the interpodal plate of first pereiopods as in female, but without the transverse ridges found in female sternal furca; fifth pereiopods similar in general form to those in *L. nordmanni* but slightly more styliform toward their tips.

Discussion

The first records known to me of *Lepeophtheirus* from *Mola mola* are those by Edwards (1840 p. 455), to which he gave the names *Caligus nordmanni* and *C. ornatus*. Baird (1850 p. 275) correctly transferred these species to the genus *Lepeophtheirus*. Stuardo (1958) examined material that he considered consisted of syntypes or paratypes of these two species from the Museum Nationale d'Histoire Naturelle, Paris, and found them to be conspecific.

Wilson (1908 p. 444) described *L. insignis* as a new species from *M. mola*. He made no attempt to differentiate this species from *L. nordmanni* and did not refer to it in later publications. A careful comparison of cotypes of *L. insignis* (U.S. Nat. Mus. Cat. No. US74370) with specimens identified as *L. nordmanni* by Stuardo revealed no significant differences and *L. insignis* must therefore be considered a junior synonym of *L. nordmanni*. Material identified by me (Hewitt 1964 p. 153) as *L. insignis* are also *L. nordmanni*.

Shiino (1960b p. 512) described a further species, *L. hastatus*, from the sunfish, and 2 years later Heegaard (1962 p. 169) described yet another, *L. molae*. A comparison of paratypes of Heegaard's species with a specimen of *L. hastatus* supplied by Professor Shiino showed that there were no significant differences and therefore *L. molae* becomes a junior synonym of *L. hastatus*. Two collections of specimens from New Zealand waters also belong to this species, which is here recorded from New Zealand for the first time.

Lepeophtheirus hastatus was clearly differentiated from *L. nordmanni* by its author. A further comparison of the two species has been made by me and the major differences are remarked on in the description above of *L. hastatus*. The most obvious of these differences are: the small accessory spine on the postantennary spine of *L. hastatus*, absent in *L. nordmanni*; the great length and styliform structure of the fifth legs in the female *L. hastatus*, these legs being short and lamelliform in female *L. nordmanni*; and the reduced, almost vestigial,

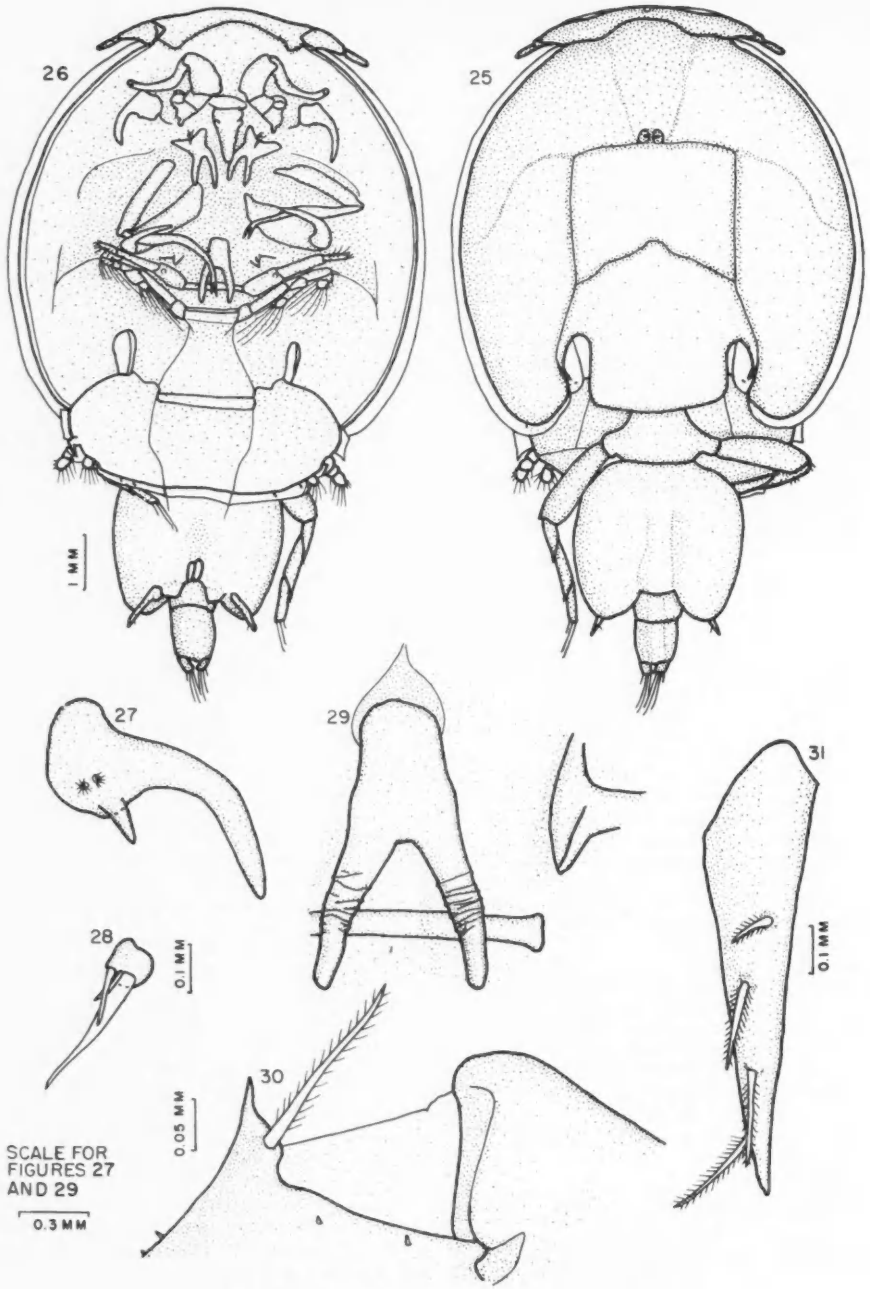

FIG. 25–31. *Lepeophtheirus hastatus* Shiino, female.
Fig. 25. Dorsal habitus; Fig. 26. Ventral habitus; Fig. 27. Postantennary spine; Fig. 28. Anterior part of first maxilla; Fig. 29. Sternal furca; Fig. 30. Fourth pereiopod, detail of joint between protopod and second segment; Fig. 31. Fifth pereiopod.

sternal furca in male *L. nordmanni*, long enough, however, to extend beyond the interpodal plate of the first pereiopods in *L. hastatus*.

Careful discrimination of these two species is required as they are similar in size, in structure of the cephalothorax, in structure of most of their appendages, and in length and striated appearance of the sternal furca of the females. There can be no doubt that they are closely related, and it is perhaps a little surprising that both Shiino and I have recorded the two species as occurring together on the same host fish. Unfortunately neither of us has recorded the location on the host. Later collections may reveal whether these two species compete in any way for position on the host.

The great similarity between the two species requires caution when dealing with previous records that do not give sufficient information to allow the species concerned to be identified from the description. On this basis, the only certain records of the two species are shown in Fig. 37 and Table 1.

It seems possible from the few records so far that *L. hastatus* may have been derived from a separate southern ocean population. Further collections of both species and more information on the wanderings of the ocean sunfish are required to shed more light on this suggestion.

The nature of the fifth leg in *L. hastatus* would suggest that it could well be included in the genus *Dentigryps* Wilson 1913, as was suggested by Lewis

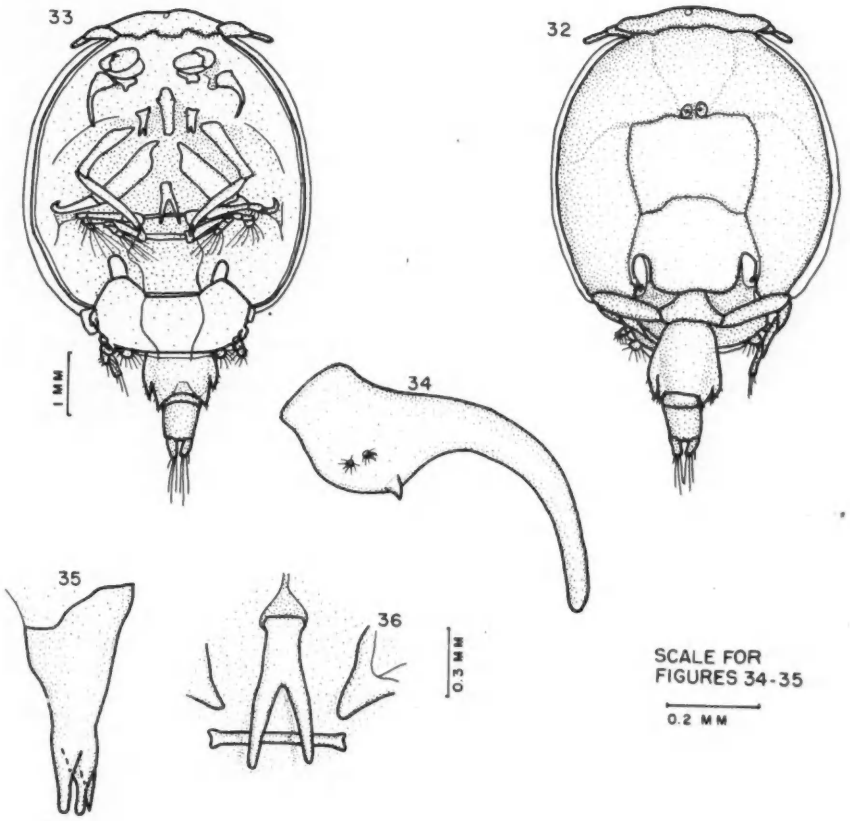

FIG. 32–36. *Lepeophtheirus hastatus* Shiino, male.
Fig. 32. Dorsal habitus; Fig. 33. Ventral habitus; Fig. 34. Postantennary spine; Fig. 35. Spinous part of first maxilla; Fig. 36. Sternal furca.

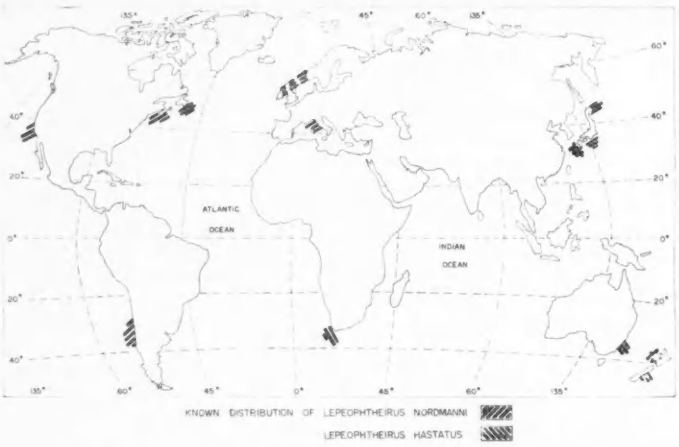

FIG. 37. Known distribution of *Lepeophtheirus nordmanni* and *L. hastatus*.

TABLE 1. World distribution of *Lepeophtheirus nordmanni* and *L. hastatus*.

Locality	General area	Source			Ident.[a]
		Lepeophtheirus nordmanni			
Nice	Mediterranean	Edwards	1840	455[b]	Spec. by Stuardo
Mediterranean	"	Heller	1865	180	Descr.
County Antrim, Ireland	North Atlantic	Baird	1850	275	Descr.
Aberdeen, Scotland	"	Scott	1900	151	Descr.
58°00′N, 0°20′E	"	Stuardo	1958	4	Spec
Woods Hole + Casco Bay, Mass.	"	Wilson	1905	623	Spec.
Woods Hole, Mass.	"	Wilson	1932	411	Descr.
Avalon Peninsula, Nfld.	"	Threlfall	1967	171	Spec.
Coast of California	North Pacific	Wilson	1908	444	Spec.
Monterey Bay, California	"	Wilson	1935	778	Descr.
Kesennuma, Miyagi Prefecture	"	Shiino	1957	359	Descr.
Shirahama, Wakayama Prefecture	"	Shiino	1959	347	Descr.
Toba, Japan	"	Shiino	1960a	481	Descr.
Valparaiso	South Pacific	Edwards	1840	455	Spec. by Stuardo
Eastbourne, New Zealand	"	Hewitt	1964	153	Spec.
Mana Is., New Zealand	"	New record			
		Lepeophtheirus hastatus			
Table Bay, South Africa	South Atlantic	Barnard	1948	247	Descr.
Seto, Japan	North Pacific	Shiino	1960b	512	Spec.
Port Jackson, N.S.W. + Botany Bay, N.S.W.	South Pacific	Heegard	1962	169	Spec.
Mana Is., New Zealand	"	New record			
Canterbury Region, New Zealand	"	New record			

[a]Ident., identified by; descr., description; spec., specimens.
[b]Page number.

(1964b p. 378) on the basis of Heegaard's description of this species under the name *L. molae*. Lewis, after a careful examination of the four species assigned to *Dentigryps*, came to the conclusion that the only major difference between this genus and *Lepeophtheirus* was in the great length of the fifth leg in *Dentigryps*. This leg reaches beyond the anal laminae in the species he examined. However, in female *L. hastatus*, this leg, when directed posteriorly, reaches to or beyond the posterior margin of the abdomen. A comparison of specimens of *L. hastatus* with specimens of *D. ulua* Lewis, kindly lent by Dr Lewis, and with Lewis's (1964a, b) description of the other species of the genus, shows that this leg is similar in form to *L. hastatus* and the species of *Dentigryps*.

The similarities betwen *L. hastatus* and *L. nordmanni* seem too many and too detailed for them to be the result of parallel evolution. It seems likely that the two species became distinct in the recent evolutionary past. The differences between the two species are not surprising since the fifth leg probably plays some part in copulation and as such would be under strong selection to differentiate once two populations that had become sibling species came into contact. An elongate fifth leg of the kind found in *L. hastatus* should then be regarded as a good character to separate species but a poor character to separate genera.

There seem to be no other characters that can be used satisfactorily to separate *Dentigryps* from *Lepeophtheirus*. Furthermore, the similarities reported by Lewis (1964b p. 375) between *D. curtus* Wilson and *D. litus* Lewis, and between *D. ulua* Lewis and *D. bifurcatus* Lewis suggest that the genus is polyphyletic in origin rather than a natural grouping.

Under these circumstances the species formerly assigned to *Dentigryps* must be regarded as somewhat unusual species of *Lepeophtheirus*.

Dentigryps curtus Wilson 1913 becomes *L. curtus* (Wilson) n. comb., *D. litus* Lewis becomes *L. litus* (Lewis) n.comb., *D. ulua* Lewis becomes *L. ulua* (Lewis) n.comb., and *D. longicauda* Cressey 1966 becomes *L. longicauda* (Cressey) n.comb. *Dentigryps bifurcatus* Lewis 1964 would become *L. bifurcatus* (Lewis) n.comb., but since this name is preoccupied by *L. bifurcatus* Wilson 1905, Lewis' species is here renamed *Lepeophtheirus lewisi* nom.nov.

Acknowledgments

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Interactions Between Some Tubificid Oligochaetes and Bacteria Found in the Sediments of Toronto Harbour, Ontario[1]

M. WAVRE AND R. O. BRINKHURST

Department of Zoology
University of Toronto, Toronto, Ont.

WAVRE, M., AND R. O. BRINKHURST. 1971. Interactions between some tubificid oligochaetes and bacteria found in the sediments of Toronto Harbour, Ontario. J. Fish. Res. Bd. Canada 28: 335–341.

Three species of tubificid oligochaetes and seven heterotrophic aerobic bacteria were identified from the sediment of Toronto Harbour, Ontario. The relative abundance of the identifiable bacteria present in the mud and in the worm feces voided after 18 hr was established using nutrient agar medium. The results indicated a reduction in the relative abundance of bacteria by about 72% and a reduction in the variety of bacteria in the feces as opposed to those detected in the mud. After a starvation period of approximately 1 week, only one type of bacteria was detected in the feces and guts of the worms, whereas all seven were shown to be ingested at one time or another. The surviving bacterium was different in each tubificid species, but *Aeromonas* sp. was seen to replace this surviving species on some occasions in all three species of worm. The results suggest that the fraction of the bacterial flora investigated forms a part of the diet of tubificids, and that differences between worm species in relation to their use of those bacteria may help to explain the survival of a number of morphologically unspecialized sediment-feeding species in close physical proximity to each other.

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STUDIES on the biology of tubificid oligochaetes have indicated that the nature of the food available to the worms may be a primary factor in determining the distribution and abundance of species (Brinkhurst and Jamieson 1971). In a preliminary study of the food available to three tubificid species obtained in Toronto Harbour, Brinkhurst and Chua (1969) demonstrated qualitative differences in the bacteria surviving passage through the gut in purely experimental conditions. The present investigation was carried out to demonstrate: (1) the degree of destruction of bacteria on passage through the gut by a simple, relatively quantitative technique; and (2) the relative abundance of the different bacteria in the feces as opposed to the food of the worms. In addition, the original experiments were repeated to confirm the result and to extend the work to include estimates of the relative abundance of the identifiable bacteria at 48-hr intervals until only a single type of bacterium could be found.

[1]Based on a thesis submitted by M.W. in partial fulfillment of the requirements for the degree of Master of Science from the University of Toronto, Toronto, Ont.

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Methods

All samples of mud and worms were collected from Hanlan's Point, Toronto Harbour (Brinkhurst 1970), using an Ekman grab or a KB corer, and samples were screened in the field using a wash bucket. The corer and wash bucket were supplied by The Wildlife Supply Company, Saginaw, Michigan. Samples of mud and lake water were taken to the laboratory intact.

Bacteria were cultured at room temperature (20–22 C) in nutrient agar (Baltimore Biological Laboratory) and seven types were identified by means of an array of morphological, biochemical, and physical tests as described by Bailey and Scott (1962), Breed et al. (1957), Cowan and Steel (1966), Gibbs and Skinner (1966), and Sneath (1960). A complete list of the diagnostic tests and the results obtained were given by Chua (MS 1969) and by Wavre (MS 1970). All the identifications were confirmed from unnamed cultures submitted to the Bacteriology Branch of the Ontario Water Resources Commission, who separated one of our cultures into two distinct entities. The seven forms used have not been attributed to species in each instance, but the same seven types have been identified by three independent workers and have been used in the laboratory now for several years. Consistent results have been obtained with the simple methods of culture employed, and the authors are confident that they are working with functional ecological units, even if these cannot be given specific names. It should also be realized that the study has deliberately been restricted to the small group of bacteria

that can be reared on nutrient agar at room temperature. Although these bacteria are derived from the same field location as the worms used, the spectrum of bacteria available as food in the natural sediment must be much greater than that used here, and the culture method undoubtedly alters the proportional representation of the bacteria that will grow under these conditions. Hence, great caution must be used in applying the results to natural situations, and they are discussed here only so far as the methodology permits.

Two methods of collecting worm feces were used in the experiments designed to demonstrate the effect on bacteria of passage through the gut. In the first, the worms were held in vials of mud inverted over collecting funnels as initiated by Alsterberg (Fig. 1) and modified by Appleby and Brinkhurst (1970). In the second, the feces were collected from an artificial interface of glass beads interposed between the mud and the overlying water in tubes held in the normal orientation. The second method was used to overcome the problems caused by worms dropping out of the Alsterberg cultures as well as by sediment drifting down into the accumulating feces.

The nature of the mud in which the worms are maintained clearly affects the rate of ingestion, but this has not been the subject of detailed investigation to date. In these experiments, every attempt was made to prepare mud in as uniform a manner as possible, and to provide a mud that was as suitable to worms as we could devise. Mud from Toronto Harbour was passed through a fine sieve (63-μ aperture), and was allowed to settle in water overnight. After 16 hr the supernatant water was carefully siphoned off and the mud was allowed to drain by setting the container on an incline. It was then ground in a mortar and pestle

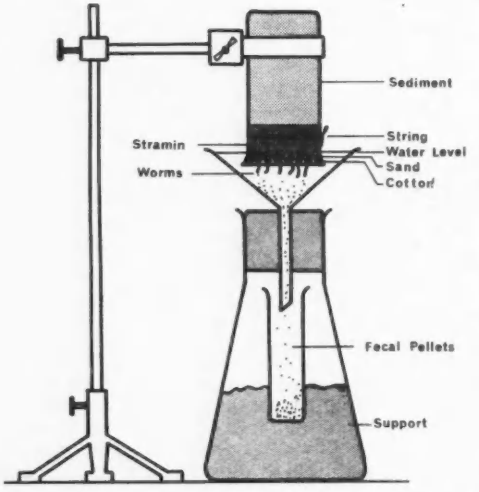

FIG. 1. Apparatus for collecting feces from inverted worm culture, the whole apparatus being immersed in a small thermostatically controlled water bath.

to break up the larger particles. The mud was then shaken up in distilled water and allowed to settle once more. Two samples of the supernatant were used to establish the number of bacterial colonies present per unit dry weight of fine suspended material (see below). The rest of the sediment was used to fill the tubes in which the worms were allowed to feed. Thirty-five worms were placed in each experimental vial, all experiments being carried out with either *Tubifex tubifex* (Müller) or *Limnodrilus hoffmeisteri* Claparède. When the worms had burrowed into the mud, a layer of sand was introduced above the mud, followed by a thin layer of cotton wool, and finally a layer of stramin, a rough burlap used in tailoring. The stramin was tightly bound to the outside of the vial to retain the contents when inverted (Fig. 1). The vials were then held in small staining jars in sterile water until the worms adopted normal orientation, with tails protruding through the stramin into the water. The vials were then inverted, held in a clamp, and collecting funnels were placed beneath the defecating worms. The whole apparatus was maintained in small thermostat baths, each of which was large enough to accommodate one experimental vial with worms and one control vial lacking them. Sterile conditions were maintained throughout the preparatory activity by exposing the equipment to a UV lamp in a sterile room. The equipment was kept in the dark and every precaution was taken to avoid undue vibration that could dislodge worms or sediment, or disturb the normal activity of the worms. Feces collected for the first hour were discarded, and those collected for the subsequent 30 min were used to establish the number of bacteria per unit weight of feces. Samples of drifted sediment were used to establish the degree of contamination of feces, although activity of the worms in the experimental vials might well increase the amount of material dislodged. No way to overcome this could be devised, but contamination of feces by sediment would increase the estimates of bacterial abundance in feces, so that gross values for percent destruction may be regarded as minimal.

The number of bacteria present in mud, feces, and drift was determined as follows. Two samples were taken of the supernatant obtained during preparation of the mud (described above) to be used as food for the worms. First, 0.1 ml was plated after 1:1000 dilution using nutrient agar, and the plates were incubated at room temperature. Plate counts were made after 48 hr. Secondly, 1.0 ml was pipetted onto a preweighed tinfoil planchet, and the sample was dried under a sun lamp for 25–30 min. The planchets were held in a desiccator prior to weighing on a Kahn electrobalance. The number of bacterial colonies per unit dry weight of suspension was estimated in this manner. The samples of drift plus feces from the experimental vials were suspended in 3 ml of distilled water, and 0.1-ml and 1.0-ml subsamples were treated as above, and the drift samples from the control vials were treated in the same manner.

In experiments using the normal orientation, the mud was prepared as before. Three vials were prepared for each experiment, one for *T. tubifex*, one for *L. hoffmeisteri*, and one without worms. When the worms had burrowed into the mud, a layer of glass beads (φ: 0.5

mm) was added to each vial instead of the sand, cotton wool, and stramin. The feces that accumulated above the glass beads were suspended in distilled water, and subsamples were taken as previously. Water samples (0.1 ml) were taken from above the glass vials of the controls both before and after the experiment. Correction factors had to be subtracted from the estimates of bacterial abundance in feces, as bacteria were present in the water over the beads in the control vials at the end but not at the beginning of the experiment. The quantity of water in feces was estimated by comparing wet and dry weights of fecal samples, and the water content was assumed to include the number of bacteria per unit volume estimated from the controls. Again, these corrections were small and consistent.

Estimates of the number and kind of bacterial species that could be obtained from feces of worms maintained for short or long periods (up to 9 days) were obtained as follows. The worms used were the same two species already named plus *Peloscolex multisetosus* (Smith). They were washed by pipetting them individually in and out of five changes of sterile water, as described by Stuart et al. (1931). Specimens of *P. multisetosus* were washed more often, as experience showed that it was difficult to dislodge sediment and bacteria from the papillate body walls of these worms. Samples (0.1 ml) of the last wash water were plated as a test of the efficacy of the washing process, and none of these produced positive growths of bacteria.

Individual worms were kept in vials containing 1.0 ml sterile saline (Lockwood 1963) in short-term experiments to establish the relative abundance and identity of bacteria in feces of worms that had previously fed in the field. In longer experiments, each worm was held in 3 ml of sterile lake water in capped vials. On the 1st, 3rd, 5th, and 7th days (and sometimes the 9th day), the water and the worms were agitated to suspend the fecal matter and 0.1-ml subsamples of the suspension were plated. At the end of the long-term experiments, the worms were washed and macerated, and samples of the gut content were suspended and plated as before.

Results

A marked reduction in the number of bacteria in feces as compared with the number in the original mud was demonstrated by both techniques (Tables 1–2). Differences in values between data derived from the Alsterberg method and those from the normal orientation technique were not significant.

A slight reduction in the diversity of bacteria was noted after passage of mud through the gut of all three tubificid species kept for 18 hr after feeding in the field, but marked changes in relative abundance were recorded (Table 3). *Flavobacterium* accounted for over half the bacterial flora of the mud, but even where its frequency of occurrence in tubificid feces was high (*P. multisetosus*) it accounted for a small percentage of the fecal microflora identified. *Pseudomonas fluorescens*, on

TABLE 1. Number of bacterial colonies per mg dry weight of sediment and of feces of *Tubifex tubifex*, cultured by Alsterberg technique and by normal orientation.

Expt.	Mud	Feces	% difference
		Alsterberg technique	
1*a*	12571	2428	81
1*b*		1080	91
2*a*	11750	2667	77
2*b*		3615	69
3*a*	10111	3352	67
3*b*		4000	60
		Normal orientation	
1*a*	12267	3760	69
1*b*		3836	69
2*a*	8739	2822	68
2*b*		2601	70
3*a*	10000	3776	62
3*b*		3791	62

TABLE 2. Number of bacterial colonies per mg dry weight of sediment and of feces of *Limnodrilus hoffmeisteri*, cultured by Alsterberg technique and by normal orientation.

Expt.	Mud	Feces	% difference
		Alsterberg technique	
1*a*	11444	2000	83
1*b*	14667	2262	85
2*a*	10785	2250	79
2*b*	11235	1560	86
3*a*	12500	2740	78
3*b*	11384	2625	77
		Normal orientation	
1*a*	12091	3707	69
1*b*		2860	76
2*a*	10917	3732	66
2*b*		3064	72
3*a*	11909	4857	59
3*b*		3614	70

the other hand, was more abundant in feces than in mud, although it was found in only half the experiments with *P. multisetosus*. The *Bacillus* species were scarce in mud and practically absent from the feces. *Aeromonas* occurred less often in feces than in mud, but when present in feces it usually accounted for a larger percentage of the identified microflora than in the mud. *Micrococcus* sp. was moderately abundant in mud but was very abundant when present in the feces of *T. tubifex* and *P. multisetosus*. It was more abundant in feces of *L. hoffmeisteri* than in mud on only a single occasion, but as it was found only twice in feces the mean percentage value remains low. *Pseudomonas* sp. occurred frequently and was then always abundant in feces of *L. hoffmeisteri*; although

TABLE 3. Relative abundance of identified bacteria present in mud and in the feces voided after 18 hr, expressed as mean percentage, and number of positive records in six experiments.

		Worm feces		
Bacterial species	Mud %–F	*T. tubifex* %–F	*L. hoffmeisteri* %–F	*P. multisetosus* %–F
Flavobacterium sp.	57.5–6	4.5–3	2.3–3	16.5–5
Pseudomonas fluorescens	7.4–6	27.6–5	43.5–5	31.6–3
Bacillus mycoides	1.3–6	0–0	0–0	0–0
Aeromonas sp.	6.4–6	8.9–2	5.2–2	20.4–4
Micrococcus sp.	13.8–6	35.7–5	7.2–2	23.8–2
Pseudomonas sp.	10.8–6	19.1–2	41.8–5	7.6–1
Bacillus cereus	2.8–6	4.2–1	0–0	0–0

TABLE 4. Relative abundance of identified bacteria (number of colonies) present in 0.1 ml of a suspension of feces of *T. tubifex* held in sterile water for 7 or 9 days (5 experiments).

Day	*Flavobacterium* sp.	*Pseudomonas fluorescens*	*Aeromonas* sp.	*Pseudomonas* sp.
1	0	0	14	38
3	0	0	31	143
5	0	0	0	197
7	0	0	0	283
1	32	0	0	64
3	21	0	0	261
5	0	0	0	252
7	0	0	0	214
1	7	0	0	54
3	21	0	0	228
5	67	0	0	209
7	21	0	0	288
9	0	0	0	291
1	0	58	0	30
3	0	144	0	129
5	0	159	0	173
7	0	53	0	257
9	0	0	0	313
1	0	0	0	45
3	0	0	0	151
5	0	0	0	261
7	0	0	0	214

it occurred less often in feces of the other two worm species, it accounted for about half the identified microflora when present.

In the long-term experiments, fecal suspension samples (Tables 4–6) showed that from two to four species were present after 24 hr, as opposed to seven initially present in the mud. Samples plated after 3–5 days showed a further reduction, only one or two species of bacteria being found in the feces in any experiment. After 7 days a single form of bacteria was found in the feces of most worms, and in the three instances where this was not so (*T. tubifex*, Table 4; *P. multisetosus*, Table 6), the second form disappeared after 9 days. The results of plating the macerated worm suspensions invariably confirmed the presence of the same bacteria as those identified in feces. In earlier tests, it had been found that the worms died soon after the bacterial flora was reduced to a single species.

In two experiments using *P. multisetosus* the surviving bacteria were identified as *Pseudomonas fluorescens*; in *T. tubifex* it was *Pseudomonas* sp. in all five experiments; and in *L. hoffmeisteri* it was *Micrococcus* sp. in four experiments. The alternate surviving species was *Aeromonas* sp., which was also found to be the survivor in some of the preliminary studies with *T. tubifex*. The other preliminary results were confirmed by the experiments reported herein. The final result may be summarized by stating that the surviving bacterium is specific to each tubificid species, but in each instance it can be replaced by *Aeromonas* sp.

The numbers in Tables 4–6 show only relative abundance of the species detected, but there is a numerical trend worthy of further comment. The bacteria surviving to days 7 or 9 are not abundant on day 1, but the numbers were higher on days 5–7. Even forms that survive only until days 3 or 5 and then disappear show a similar increase in relative abundance at first (Tables 4–6). In the single experiment with *L. hoffmeisteri* in which *Aeromonas* sp. survived (Table 5), it was abundant on day 1 but *Micrococcus* sp., which survived in the four other experiments, was absent. With *P. multisetosus* the survival of *Pseudomonas fluorescens* or *Aeromonas* sp. remained open to doubt until the 7th or even 9th day, which suggests a competitive interaction between them.

The data are summarized in slightly different form in Table 7, in which the frequencies of occurrence of all bacteria in all three worms are compared. The number of species decreased from day 1 to day 9, but the number of experiments in which a single species remained invariably increased, reaching 5 by day 7 or 9.

TABLE 5. Relative abundance of identified bacteria (number of colonies) present in 0.1 ml of a suspension of feces of *L. hoffmeisteri* held in sterile water for 7 days (5 experiments).

Day	*Flavobacterium* sp.	*Pseudomonas fluorescens*	*Aeromonas* sp.	*Micrococcus* sp.	*Bacillus cereus*
1	0	0	0	33	26
3	0	0	0	129	0
5	0	0	0	231	0
7	0	0	0	264	0
1	0	0	85	16	0
3	0	0	172	98	0
5	0	0	32	201	0
7	0	0	0	264	0
1	0	25	116	0	0
3	0	97	235	0	0
5	0	0	217	0	0
7	0	0	301	0	0
1	0	0	98	27	0
3	0	0	0	241	0
5	0	0	0	271	0
7	0	0	0	285	0
1	31	9	89	31	0
3	0	38	0	159	0
5	0	58	0	219	0
7	0	0	0	191	0

Discussion

In the preliminary study by Brinkhurst and Chua (1969), one bacterial species survived passage through the gut of each worm species after 1 week. The pattern of the results of all experiments to date shows that a single species of bacterium is the characteristic survivor for each worm species (*Micrococcus* sp. for *L. hoffmeisteri*, *Pseudomonas* sp. for *T. tubifex*, and *Pseudomonas fluorescens* for *P. multisetosus*), but that *Aeromonas* sp. can replace any of these in any given experiment. *Chromobacterium* sp., present in the sediment from September to April, is missing during the summer when it is replaced by *Aeromonas* sp. In April 1967, *Chromobacterium* and *P. fluorescens* were found together in the gut of *P. multisetosus*, but the experiments were not continued in order to determine the ultimate survivor. It is possible that both bacteria would have survived for long periods, but in all other experiments in which two bacterial species survived to day 7, only one remained on day 9. Further winter studies must be carried out to determine the survival of *Chromobacterium* sp.

The disappearance of bacterial species, even in such artificial conditions, and the degree of destruction of bacteria on passage through the gut, indicate that these bacteria are a primary food source for the worms, as they are for other benthic sediment-feeders (Zobell and Feltham 1938; Rodina 1963; Zhukova 1963; Marzolf 1964; Newell 1965; Satchell 1967; Hargrave 1970).

The differences between the worm species observed to date in this study and in those studies based on defecation rate (Appleby and Brinkhurst 1970) are consistent with the view of Brinkhurst and Chua (1969) that partitioning of the nutritional resources may provide a mechanism by which three or more unspecialized detritus feeders are able to coexist despite continuous sharing of the same sediment.

TABLE 6. Relative abundance of identified bacteria (number of colonies) present in 0.1 ml of a suspension of feces of *P. multisetosus* held in sterile water for 7 or 9 days (5 experiments).

Day	*Pseudomonas fluorescens*	*Bacillus mycoides*	*Aeromonas* sp.	*Bacillus cereus*
1	39	0	136	0
3	53	0	208	0
5	147	0	163	0
7	209	0	0	0
1	141	2	157	0
3	26	0	219	0
5	120	0	150	0
7	26	0	219	0
9	0	0	201	0
1	17	0	120	0
3	65	0	206	0
5	119	0	235	0
7	251	0	0	0
1	0	0	134	3
3	0	0	235	0
5	0	0	197	0
7	0	0	188	0
1	31	0	203	0
3	68	0	309	0
5	0	0	197	0
7	0	0	183	0

TABLE 7. Frequency of occurrence of identified bacteria in the feces of worms at intervals from days 1–7 or 9, summarized from Tables 4–6. Data represent a summary of five experiments for each worm species.

	T. tubifex					*L. hoffmeisteri*				*P. multisetosus*				
Day:	1	3	5	7	9	1	3	5	7	1	3	5	7	9
Flavobacterium sp.	2	2	1	1	0	1	0	0	0	0	0	0	0	0
Pseudomonas fluorescens	1	1	1	1	0	2	2	1	0	4	4	3	3	2
Bacillus mycoides	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Aeromonas sp.	1	1	0	0	0	4	2	2	1	5	5	5	3	3
Micrococcus sp.	0	0	0	0	0	4	4	4	4	0	0	0	0	0
Pseudomonas sp.	5	5	5	5	5	0	0	0	0	0	0	0	0	0
Bacillus cereus	0	0	0	0	0	1	0	0	0	1	0	0	0	0
Total no. of bacterial forms in all five expt.	4	4	3	3	1	5	3	3	2	4	2	2	2	2
Total no. of expt. with a single form of bacteria present (from Tables 4–6)	1	1	3	3	5	0	2	3	5	0	1	2	4	5

The differences between the relative abundance and diversity of bacteria in mud and in feces deposited by worms immediately after feeding in the field suggest that the worm population affects the composition of the microflora as well as being affected by it. However, we will be unable to combine data on relative abundance of worms, their defecation rate, the division rate of bacteria, and their rate of destruction by worms, in order to obtain a dynamic picture of this interaction, until it is possible to work with truly quantitatives estimates of the bacterial populations.

Acknowledgments

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Effects of Salinity on the Survival and Growth of Pre-Smolt Coho Salmon (*Oncorhynchus kisutch*)

ROBERT G. OTTO[1]

Department of Biology, University of Victoria, Victoria, B.C., and Fisheries Research Board of Canada, Biological Station, Nanaimo, B.C.

OTTO, R. G. 1971. Effects of salinity on the survival and growth of pre-smolt coho salmon (*Oncorhynchus kisutch*). J. Fish. Res. Bd. Canada 28: 343–349.

Salinity tolerance of juvenile coho salmon (*Oncorhynchus kisutch*) increased markedly during the period from approximately 1½ months after emergence from the gravel to the onset of the smolt transformation, except for a decline in the fall. In January, salinity tolerance ceased to limit dispersal to the sea. The limiting effects of high salinities on survival were less for larger fish than for smaller individuals and were substantially reduced by a period of exposure to dilute salinities. Growth rate, food intake, and gross food conversion efficiency had the highest values at salinities of 5–10 ppt throughout the pre-smolt period. The results are discussed in relation to the feasibility of using saltwater impoundments as a management tool in increasing coho production.

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IN this study, salinity tolerance and the effects of increased salinity on growth were investigated in juvenile coho salmon (*Oncorhynchus kisutch*) that had not undergone smolt transformation, a physiological phenomenon associated with preadaptation for marine life (Hoar 1958). Many coho emigrate to the sea upon transformation; however, a major proportion of the hatch emigrates shortly after emergence from the redd (April–June) and lesser numbers go to sea throughout the remainder of the pre-smolt period. This presmolt emigration is believed to result from intraspecific competition for food and space (Chapman 1962; Mason and Chapman 1965). Records compiled by Chapman (1962) for a number of Washington and British Columbia streams indicate that the number of pre-smolt emigrants greatly exceeds that of smolts in many instances. Evidently half or more of the total emigrants are presmolt, fish that have not undergone the physiological preadaptation for marine life. The ability of the presmolt emigrants to adapt to increased salinities is therefore of interest, particularly in view of their potential contribution to the fishery.

Materials and Methods

The experiments began about 1½ months after emergence of fry from the redd. The tolerance tests were conducted on five occasions during the pre-smolt period (1967–68, Table 1) and the growth studies on four occasions (Fig. 6). Fish used were collected as fry from the upper Cowichan River, Vancouver Island, B.C. They were transported in aerated cans to holding facilities at the Fisheries Research Board of Canada, Biological Station, Nanaimo, B.C.

All tests were performed at 10 ± 0.1 C. Salinities were determined by the modified Mohr method as described by Strickland and Parsons (1965) and were accurate to ±1.0 ppt. For the tolerance tests, a synthetic sea salt mixture (Rila Marine Mix, Rila Products Inc., Teaneck, N.J.) was added to fresh water. For growth studies, low salinities were obtained by diluting sea water with fresh, and salinities above that of available sea water were obtained by adding synthetic sea salt to sea water.

The tolerance tests were conducted under continuous light. A natural day length was maintained for the growth studies.

SALINITY TOLERANCE

Salinity tolerances were tested in fiberglass tanks of 50-liter capacity. Temperatures were regulated in each tank by balancing a thermostatically controlled heater against a continuous cooling system. Aerators in each tank circulated the water, preventing stratification. Test fish were restricted to baskets of nylon netting to isolate them from the temperature-control apparatus.

For each test series, groups of 10 fish were placed in the experimental tanks containing fresh water. The fish were temperature-acclimated at a rate of 1 degree C per day before use. Then a 24-hr adjustment period was allowed after which one-third of the water was siphoned from each tank and replaced with a salt solution of sufficient concentration to raise the tank salinity to the desired level.

The fish were observed for 7 days. Individual times to death were plotted in cumulative rectilinear form

[1]Present address: Department of Zoology, Arizona State University, Tempe, Ariz. 85281, USA.

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as described by Bliss (1937 p. 821). Progressive mortality on a probit scale was plotted against the logarithm of time to death in minutes. The median resistance time, that time required for 50% of the test fish to die, was obtained from the line fitted by eye to the probit plot and used for comparisons between tests. To ensure that the 7-day test period was adequate for all fish to react, fish from the July test series that survived the normal 7-day test were held for an additional 23 days, during which no further mortality occurred. For a measure of the lethal concentration, the upper incipient lethal salinity (Fry 1947) for each test period was estimated by graphing the 50% survival times against salinity. This value is the concentration at which 50% of the test population would be expected to survive the effects of salinity for an indefinite period.

TABLE 1. Survival on various dates for groups of 10 freshwater-adapted pre-smolt coho salmon exposed to various salinities at 10 C (1967–68).

Test salinity (*ppt*)	Standard length (*cm*)	Total mortality (%)	Median survival time (*min*)
	May 20–26		
10.0	3.22	0	[a]
13.0	3.31	0	[a]
16.0	3.29	0	[a]
19.0	3.23	0	[a]
22.0	3.35	30	[b]
25.0	3.34	80	2320
28.0	3.37	100	1710
31.0	3.25	100	630
33.0	3.15	100	347
	July 19–25		
17.5	4.44	0	[a]
20.0	4.46	0	[a]
22.5	4.43	40	[b]
25.0	4.46	60	5200
27.5	4.42	90	2980
30.0	4.43	100	1700
	Oct. 15–22		
17.5	5.17	0	[a]
20.0	5.10	10	[b]
22.5	5.09	20	[b]
25.0	5.10	90	2800
30.0	5.22	100	1240
35.0	5.15	100	720
40.0	5.06	100	480
45.0	5.00	100	335
	Dec. 26–Jan. 1		
25.0	7.10	0	[a]
30.0	7.28	50	3700
35.0	7.23	100	1125
40.0	7.20	100	680
45.0	7.05	100	410
	Feb. 3–9		
25.0	7.88	0	[a]
30.0	7.83	30	[b]
35.0	7.85	100	1300
40.0	7.86	100	765
45.0	7.86	100	400

[a]Survival in the 7-day observation period was 100%.
[b]Distribution of response times was truncated.

Results for a single test series (October 15–22) are shown in Fig. 1. Logarithms of individual times to death are plotted in cumulative fashion for groups of 10 fish exposed to 20, 22.5, 25, 30, 35, 40, and 45 ppt. No mortality occurred in a test conducted at 17.5 ppt. For this series, the upper incipient lethal salinity would be approximately 23 ppt. Total mortality occurred in the following times: 45 ppt, 475 min; 40 ppt, 665 min; 35 ppt, 1180 min; 30 ppt, 1850 min. 100% mortality did not occur at salinities below 30 ppt and the lowest salinity at which total mortality would be expected is between 25 and 30 ppt. Conversely, the maximum salinity at which total survival would be anticipated to occur is between 17.5 and 20 ppt. However, because of the high variance associated with estimates of conditions for 100 or 0% mortality, the upper incipient lethal level as employed is considered a more useful method of estimating and comparing tolerance.

GROWTH

The experimental tanks described for tolerance tests were used for the growth tests also. To avoid competitive problems, a Plexiglas divider was used to break the tanks into two sections.

Two weeks before the beginning of a test series, approximately twice the required number of fish were placed in laboratory holding tanks for acclimation to the experimental temperature and diet of frozen brine shrimp (*Artemia*). Test groups of 10 fish each were then

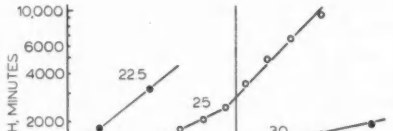

FIG. 1. Log-probit distributions of time to death for freshwater-adapted pre-smolt coho exposed in groups of 10 to salinities from 22.5 to 45 ppt at 10 C. Distributions were fitted by eye.

selected by assigning individual fish to buckets according to a table of random numbers. Very large or very small fish were excluded to avoid competitive problems. The fish were not fed for 24-hr before selection. Once in the experimental tanks, they were allowed a 12- to 18-hr period to adjust to their new surroundings. Feeding began the following morning. The fish were fed to satiation twice daily.

Weights and lengths of all fish were recorded immediately before and at regular intervals during each experimental period. Fish were anesthetized individually in tricaine methanesulfonate (M.S. 222); standard length was measured (±0.5 mm) and the fish were then blotted on damp filter paper and weighed (±0.02 g). After weighing they were returned to the experimental tanks.

At the beginning of each series, a sample of 10 fish from the stock group was used to estimate the initial percent dry weight. The fish were oven-dried at 38 C to minimum weight. A similar procedure was followed at the end of each series for all test groups.

To inhibit the accumulation of metabolites and bacterial growth, the water in each tank was changed and the tank cleaned every 4th day. In addition, approximately a third of the water in each tank was replaced every 2nd day.

Salinity Tolerance

Distinct seasonal changes in the adaptive capacity of juvenile coho to salinity were observed (Fig. 2). Median survival times increased during the summer, declined somewhat during the fall, and then rose again during the winter and spring as the smolt transformation began and the migration period approached. The upper incipient lethal salinity followed a similar seasonal progression of change, increasing from 22–25 ppt from May through October to 25–30 ppt in December and to 30–35 ppt in February. The fish were clearly incapable of surviving an abrupt movement from the parent stream to full strength sea water during the early portion of the pre-smolt period. These results conflict somewhat with those obtained earlier by Conte et al. (1966) who observed survival of various samples of juvenile coho exposed to 30 ppt to range from 0 to 100% during the same period. However, comparison of results is difficult since the fish used by these authors were hatchery-reared, of a larger size, and not subject to controlled temperatures.

Effects of size on tolerance to high salinity were tested from November 12 to 18 on seven groups of coho fry selected according to length (Fig. 3). Survival times of juvenile coho at 40 ppt were directly related to size. Median survival time of the largest group of fish was more than twice as great as that of the smallest. Since ages of the individual fish were not known, no inference can be made between age and median survival time. It seems reasonable to assume, however, that the size differences at the time of testing resulted primarily from factors other than time of hatching.

To determine the effect of acclimation to dilute salinities on tolerance, groups of 10 fish were maintained in fresh water, 5, 10, 15, and 20 ppt salinity from August 8 to September 12. After the 35-day acclimation period, all groups were transferred to 34 ppt salinity for salinity tolerance tests.

Salinity tolerance was clearly increased by exposure to dilute salinities, the increase in tolerance being directly related to the concentration (Fig. 4).

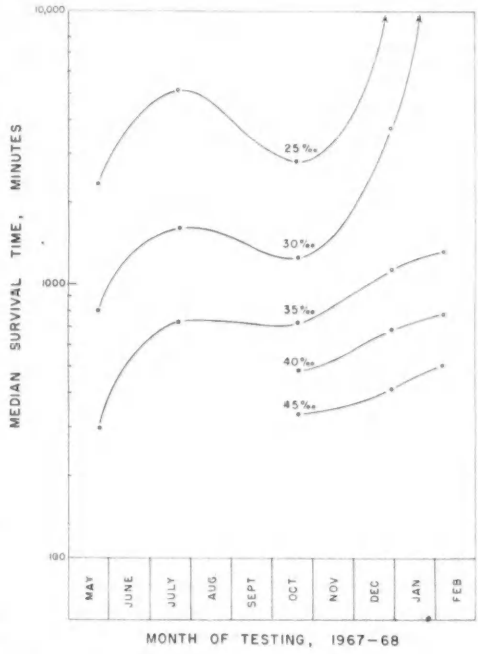

FIG. 2. Median survival times for groups of 10 fresh-water-adapted pre-smolt coho exposed to high salinities at 10 C.

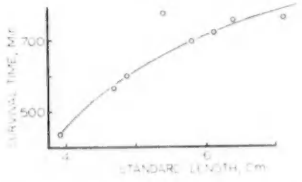

FIG. 3. Median survival times for groups of 10 fresh-water-adapted pre-smolt coho of various sizes exposed to 40 ppt salinity for 7 days at 10 C.

Mortality for the 7-day period ranged from 100% for those fish acclimated to fresh water to only 10% for fish acclimated to 20 ppt salinity.

Growth

To simplify the description of salinity effects on growth, mean increases in dry weight were transformed to relative weight increases using a common initial value of 1.000. The cumulative relative weight increases were plotted against time and salinity (Fig. 5). From June to September, salinities below 10 ppt had little effect on the total increase in weight. At salinities above 10 ppt, however, growth was inhibited. In November and January a distinct ridge occurred in the response distribution at a salinity of 10 ppt; this ridged configuration resulted primarily from a relative decrease in the magnitude of response in fresh water and at 5 ppt rather than an increase at 10 ppt. Of primary interest in the series of tests are: the consistent maximization of growth at intermediate salinities of 5–10 ppt, the gradual seasonal decline in the rate of weight increase for fish maintained in fresh water in relation to that of fish at higher salinities, and the marked reduction in growth rate of fish maintained at salinities greater than 10 ppt throughout the test period. Also, throughout the pre-smolt period the salinities at which the greatest growth occurred corresponded closely with the preferred salinity as demonstrated by Otto and McInerney (1970).

Growth Rate

The mean daily percent increases in weight were not measurably different for salinities of 10 ppt and below from June to September (Fig. 6). Above 10 ppt, growth rate was depressed. From November to February, the rate of growth was highest at 10 ppt, the general pattern of response at salinities above 10 ppt being similar to that noted previously. However, growth rate of fish maintained in fresh water declined after September so that by February, the mean rate of growth in fresh water was only slightly greater than that observed at 15 ppt and above.

A precipitous decline in the rate of growth at all test salinities was observed in January. At this time, heavy construction work outside the building resulted in high noise and vibration levels in the laboratory and the fish were reluctant to feed.

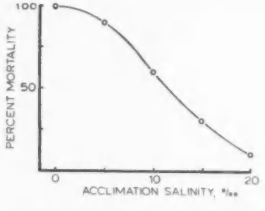

FIG. 4. Percentage mortalities for groups of 10 pre-smolt coho exposed to 34 ppt salinity for 7 days after being held at various salinities for 35 days (10 C).

Food Intake

Environmental salinity markedly influenced the food intake of juvenile coho (Fig. 7). In June, the daily food intake was highest in fresh water, decreased slightly at 5 and 10 ppt, and dropped sharply at 15 ppt. By August, the modal response had shifted to 5 ppt and the food intake at 15 ppt was much improved over that noted for the previous test series. However, those fish maintained at 20 ppt clearly had a depressed appetite. In November and January, the highest food intake occurred at 10 ppt. The daily ration at salinities above 10 ppt was again reduced.

Daily food intake showed a consistent decline with increasing age or body size, or both. In June the fish in fresh water consumed an average ration of about 16% of their body weight per day. Ration size then decreased to 13% in August, 11% in November, and 7% in January for this group. A similar reduction in food intake occurred at other salinities.

Gross Food Conversion Efficiencies

Gross food conversion efficiency was measured as the percent increase in dry weight per unit intake of dry ration. Mean efficiencies were computed for each 30–33-day period (Fig. 8). Highest values for food conversion efficiency were observed at salinities of 5 and 10 ppt in all test series. The lowest efficiencies were observed at 15 ppt except in November, when those fish maintained at 25 ppt (the approximate upper lethal salinity) had the lowest value.

From September to January, the conversion efficiency of those fish maintained in fresh water declined in relation to that of other test groups, indicating that the observed reduction in growth rate during this period resulted from both a reduced efficiency of food conversion and a decrease in food intake.

Conversion efficiency throughout the year was low, suggesting that frozen brine shrimp constitute a poor experimental diet. F. P. Conte (personal communication) has suggested that frozen brine shrimp are nutritionally inadequate and may produce dietary deficiencies in juvenile salmon

if fed exclusively. During the relatively short experimental periods in these tests no external signs of such dietary deficiencies were noted. All fish appeared healthy and remained active throughout the test periods. Growth rates for fish in both the holding tanks and experimental tanks (fresh water) closely approximated that observed by the author (unpublished data) for wild fish in the Cowichan River.

Discussion

The results of this study show that underyearling coho are poorly equipped physiologically to survive emigration to the sea before smolt transformation. Accordingly, pre-smolt emigrants would not be expected to contribute substantially, if at all, to the fishery for adult salmon even though they may in some instances (Chapman 1962) outnumber

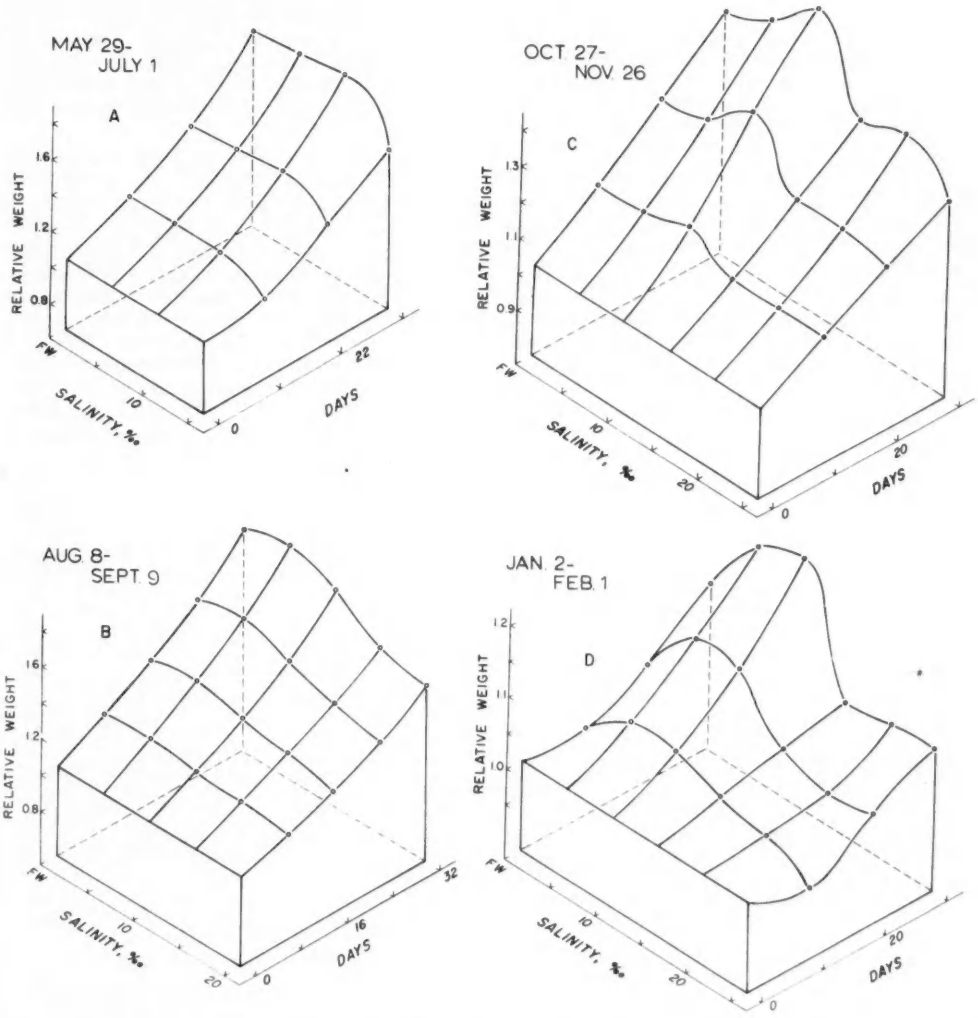

FIG. 5. Mean relative weights for groups of 10 pre-smolt coho maintained at various salinities at 10 C. The data were plotted relative to an initial weight of 1.000 and the curves fitted by eye.

those fish that emigrate as smolts. Scale pattern diversity of the type expected with differing ages of seaward emigration (as for example in sockeye salmon (Forester 1968 p. 381)) has not been reported for coho, thus providing indirect support for this conclusion.

Such a conclusion must, however, be tempered by several related considerations. This study proceeded on the assumption that there are no genetic differences with respect to sea water tolerance between pre-smolt and smolt emigrants. This is consistent with the results of Chapman (1962) and Mason and Chapman (1965), suggesting that it is intraspecific competition that initiates the pre-smolt emigration. The possibility cannot be excluded, however, that the experimental stock may not have been representative of pre-smolt emigrants genetically.

The characteristics of the environment at the mouth of the parent stream may also be of importance. Where there are extensive estuarine areas available, survival might be possible since it has been shown that juvenile coho have the ability to select salinities within the range of tolerance and, indeed, prefer those salinities where growth, food intake, and food conversion efficiency are maximized (Otto and McInerney 1970). The ability to survive at higher salinities after acclimation as shown in this study is an additional variable favoring survival, where the dimensions of the estuary and the ability of the fish to remain within the estuary are sufficient to accomplish the acclimation process. Also, as the season advances or as the pre-smolt emigrants become larger, or both, the chances of survival are improved as salinity tolerance increases. Working against this potential size advantage is the fact that pre-smolt emigrants are usually the smaller individuals (Chapman 1962).

From a practical standpoint, the possibility of culturing underyearling coho in an estuarine environment is attractive on several counts. Favorable, dilute salinities could be maintained for fish, predominantly pre-smolt emigrants that may otherwise be lost to the fishery. Rapid acclimation in conjunction with the enhanced growth capacity at dilute salinities as shown in this study and by Canagaratnam (1959) and the high food production capabilities of estuarine systems would probably mean that the fish could be released in a few weeks to continue their seaward migration. Additional support for the potential of estuarine culture is found in a series of studies conducted by the Oregon Game Commission (Garrison 1965). As an example, their results show that in March of 1964, 86,000 coho fry were introduced into a tidal estuary in which salinities varied from fresh to full sea water. At the end of 3 months, 26,000 "smolts" were

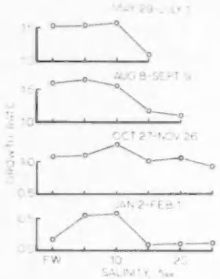

FIG. 6. Mean growth rates as daily percent increases in weight for groups of 10 juvenile coho maintained at various salinities at 10 C.

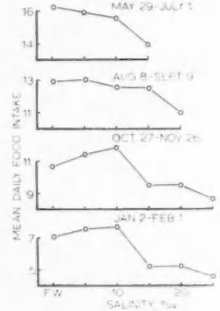

FIG. 7. Mean daily food intakes as percents of dry body weights for groups of 10 pre-smolt coho maintained at various salinities at 10 C.

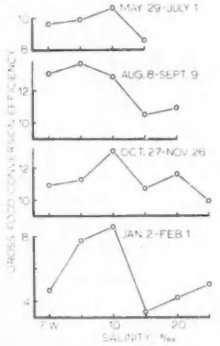

FIG. 8. Mean gross food conversion efficiencies as percent increases in dry weight per unit intake of dry ration for groups of 10 pre-smolt coho maintained at various salinities at 10 C.

removed from the saltwater end of the estuary averaging 12.3 cm and 19.6 g. Both the growth and physiological changes that normally require 1 year were accomplished in a period of 3 months.

Acknowledgments

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Relative Rates of Utilization by Commercial and Sport Fisheries of Atlantic Salmon (*Salmo salar*) from the Miramichi River, New Brunswick

C. J. KERSWILL[1]

Fisheries Research Board of Canada
Biological Station, St. Andrews, N.B.

KERSWILL, C. J. 1971. Relative rates of utilization by commercial and sport fisheries of Atlantic salmon (*Salmo salar*) from the Miramichi River, New Brunswick. J. Fish. Res. Bd. Canada 28: 351–363.

Studies of naturally produced Atlantic salmon (*Salmo salar*) smolts, marked from 1950 to 1961 on tributaries of the Miramichi River by differential finclipping, indicated that commercial fisheries accounted for over 60% of their total utilization as adults. The bulk of commercial recoveries was about equally divided among Newfoundland traps, Miramichi drift nets, and Miramichi trapnets; the remainder came from various commercial catches in the Maritime Provinces and Quebec. The percentages of grilse (1 sea-year fish) in commercial catches were low, 20% for Northwest Miramichi and 17% for Southwest Miramichi fish.

Anglers reported 10 and 7%, respectively, of all the recaptures of Northwest and Southwest fish. All these angling recoveries were made in freshwater sections of the Miramichi River. Grilse comprised over 70% of angling recaptures.

Spawning escapement accounted for 25% of the Northwest returns, of which 60% were grilse; and 19% of the Southwest returns, of which 85% were grilse.

For the total of 174,509 smolts marked on the Northwest Miramichi the minimum survival rate to fisheries and spawning escapement was 2.7%. For 78,117 Southwest smolts, the corresponding value was 2.0%. These survival rates, except for the Newfoundland commercial catches, were not adjusted for completeness of search and other factors.

The average ratio of grilse to larger salmon in the adult recoveries, about 0.6:1, was much lower than in more recent smolt-tagging experiments by another worker (about 2:1) and indicated a considerable increase in the ratio for Miramichi stock between 1950 and 1968.

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IN 1949 the Government of Canada and the governments of the Atlantic Provinces set up a coordinating committee to investigate the factors limiting production and utilization of Atlantic salmon (*Salmo salar*) and to improve management of the fisheries. The stimulus was a steady decline since the early 1930's in the commercial catches in tidal waters of the Canadian Atlantic coast, and concern expressed by anglers over the future of the sport fisheries in freshwater sections of the rivers (Menzies 1951).

As part of the resulting expansion of salmon investigations under the Fisheries Research Board of Canada, a smolt marking and recovery program was begun in 1950 on the Miramichi River, which supports extensive commercial and angling fisheries. The main objective was to determine the contributions of Miramichi-produced salmon to the commercial and sport fisheries of various areas and the stocks available for spawning escapement. Earlier work on Miramichi salmon comprised mainly life-history studies based on scale reading (Huntsman 1931). Other smolt production studies in eastern Canada involved much smaller rivers, for example the Margaree in Nova Scotia (White 1939) and the Petitcodiac in New Brunswick (Elson 1962). The relation of such studies to other research aimed at managing the Atlantic salmon resource was discussed by Kerswill (1961).

From 1950 to 1961 salmon smolts were marked by finclipping as they descended Miramichi tributaries. This paper analyses data on recaptures of marked adults from 1952 to 1964 from sport and commercial fisheries as well as from research-operated sampling traps, counting fences, and inspection of wholesale dealers' stocks. Fishery officers, fishermen, and camp owners throughout the Maritimes and Newfoundland were alerted to the importance of submitting returns on finclipped salmon.

During recent years Miramichi anglers have become concerned over an increase in the percentage of grilse (1 sea-year fish) in their salmon catches.

[1]Present address: Fisheries Research Board of Canada, Sir Charles Tupper Building, Ottawa 8, Ont.

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For the Northwest Miramichi this is reflected in the records of ascending fish at a counting fence near the lower end of the angling area from 1950 to 1969 (Fig. 1). From 1950 through 1961 the percentage fluctuated around an average of about 70% but in all the following 8 years it exceeded 90%.

The Salmon Fisheries of the Miramichi

The Miramichi River (Fig. 2) flows from a little west of the centre of New Brunswick in a generally easterly direction into Miramichi Bay on the east coast, thence into the Gulf of St. Lawrence. Its oval watershed is roughly 100 miles long, 80 miles wide, and 4500 square miles in area or about one-sixth the area of the province. Numerous tributary streams feed the two main branches, namely the Northwest Miramichi and the Main Southwest Miramichi rivers. Both branches are tidal for about 15 miles before joining near Newcastle to form a common estuary. About 15 miles farther east the common estuary widens into Miramichi Bay.

Practically the whole Miramichi system is accessible to adult salmon entering from the sea. Such falls as exist impede upstream movement only occasionally, at times of extreme water conditions. Only two minor tributaries have dams and that on the largest, the Bartholomew River, is provided with a fishway. Gravel suitable for spawning is generally abundant, and young salmon are found in nearly all the tributaries. Much of the watershed is densely wooded and most of the upper branches were inaccessible by car before 1950. In recent years balsam fir and spruce stands in many upriver areas have been cut extensively for pulpwood; also, springtime stream driving of pulpwood and large logs has been replaced by trucking. Before 1954 pollution affecting salmon was virtually unknown here. In 1954 aerial insecticide spraying was introduced to control an outbreak of the spruce budworm and it has continued annually except in 1959 (Elson and Kerswill 1964; Kerswill 1967). Since 1960 heavy metal pollution from copper and zinc mining operations has affected salmon behaviour and survival on the Northwest Miramichi River (Saunders and Sprague 1967).

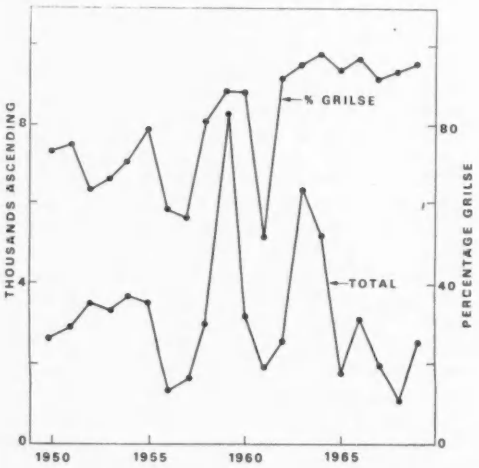

FIG. 1. Total numbers of ascending Atlantic salmon (grilse and larger salmon) trapped at Curventon counting fence, 1950–69, and percentages of totals that were grilse. Data for 1950–68 from Saunders (1969); for 1969, from unpublished data.

The Miramichi has the highest Atlantic salmon angling catch of all Canadian rivers. From 1949 to 1963 it ranged from 14,000 to 58,000 fish per year and averaged about 30,000 fish (Anonymous 1964). This comprised about 60% of the total catch in all rivers of the provinces of New Brunswick and Nova Scotia.

Fixed trapnets are scattered along the shores in tidal waters of the estuary from near tidehead to outer Miramichi Bay. In 1950 the number licensed totalled 160 and since then has decreased. Catch statistics are given as weights and, on the basis of an average weight of 10 lb per fish, the total Miramichi trapnet catches from 1953 to 1963 ranged between 6500 and 31,000 fish and averaged 13,000 per year.

A fleet of about 130 drift-net boats operates in the Gulf of St. Lawrence outside Miramichi Bay, going 25–30 miles offshore on 5 nights per week during a 10- to 11-week summer period. Bad weather often prevents fishing. At Escuminac and nearby ports from 1953 to 1963 a resident technician recorded daily the numbers of fish taken by drift nets. Total annual catches ranged from 6800 to 31,000 fish and averaged 17,000 per year. A tagging experiment (Belding and Prefontaine 1939) showed only part of the drift-net catch to be of Miramichi origin.

The angling fishery in Miramichi freshwater areas can legally take salmon of any size or age that have spent at least one winter in the sea after leaving fresh water as smolts (2–4 years old; 11–18 cm long) during May and June of previous years. Mature salmon returning to rivers after only one winter at sea are called "grilse." The grilse are typically 45–65 cm long and weigh 2½–6 lb (1.1–2.7 kg); after two or more winters at sea the fish

are called "large salmon" or just "salmon" and usually weigh 8 lb (3.6 kg) or more.

The commercial fishery in the Miramichi as in other New Brunswick areas is restricted by regulation to capture of salmon weighing 5 lb and over, though the general federal regulation for Atlantic marine areas prohibits only the taking of salmon under 3 lb in weight. In the Maritime Provinces the commercial catch is predominantly large salmon but considerable quantities of grilse may be and are taken in many Atlantic coast areas.

Methods

Source and Handling of Smolts

The smolts for marking were obtained at counting fences: (*a*) on the Northwest Miramichi River from 1950 to 1961 at Curventon 7 miles above head-of-tide, and from 1958 to 1961 at Camp Adams 33 miles above Curventon; (*b*) on the Southwest Miramichi from 1950 to 1957 at Pineville on the Dungarvon River, $\frac{1}{4}$ mile above its confluence with the Renous River and 6 miles above head-of-tide, and from 1958 to 1961 at Shinnickburn on the Cains River, 25 miles above head-of-tide.

The counting fences were temporary structures installed each spring as soon as the spring freshet had subsided enough to permit work. They consisted of wooden pole A-frames set about 7 ft apart, which supported straining racks placed against their upstream sides. Arms of the fence set at an angle with the stream banks led descending smolts towards simple square or rectangular traps. The general plan is described by White (1939) for a small installation on the Margaree River. All counting fences except those on the Cains also had up-traps for ascending fish. The arrangement of fence arms and traps varied with the site, but the aim was to use the widest possible part of the stream and give maximum water straining area to relieve pressure during freshets.

At the Curventon fence (Fig. 3*a*) the river width is 315 ft, the bottom is fairly level sandstone bedrock, and the water depth ranges from a few inches at midsummer low to 4–5 ft during extreme freshets. In 1950–52 there was one trap for descending fish near the west bank; from 1953 on, there were traps near either shore for descending fish and one trap near midstream for ascending fish. The straining surface was a row of separate heavy wooden frames $2\frac{1}{2}$ ft wide and about 5 ft high. Quarter-inch diameter steel rods were positioned vertically across the face of each rack with $\frac{3}{4}$-inch spacing. The supporting A-frames, at 7-ft intervals, were held together by spruce poles. Sand bags covered spaces between

FIG. 2. Eastern Canada showing locations of places mentioned in text; Miramichi smolt-marking sites indicated by solid black circles in the inset.

river bottom and racks, and either alone or with sand-filled boxes provided ballast. Additional support was provided by ¾-inch steel cables attached to the rear legs of the A-frames and running forward several feet to steel pins set in holes drilled into the bedrock. The up- and down-trap boxes were 8 × 10 ft in area by 6 ft high; they had board floors and tops, and the sides were similar to fence panels, with ½-inch steel rods set vertically with ⅝-inch spaces.

At the Dungarvon site (Fig. 3*b*) the river is 150 ft wide and single up- and down-traps were used, as at Camp Adams (Fig. 3*c*) where the width is 100 ft. The Cains River at the trap site is about 250 ft wide with an island near the middle; a fence was installed only from the north bank to the island to provide a sample of the smolt run (Fig. 3*d*).

Installation and maintenance of traps and handling of fish were the responsibility of full-time scientific and technical staff assisted by university students in the summer. Traps were cleared of fish day or night as required, to interfere as little as possible with ascent or descent and to avoid overcrowding. Prolonged spring freshets and drives of logs and pulpwood sometimes caused postponement of trap installation until the peak of the smolt run had passed. In some years small numbers, but usually many thousands, were finclipped.

The fins were removed at the level of the skin surface with steel wire clippers, the bevel of the undersides of the blades having been filed off. The smolts were not anaesthetized; they were simply picked up and held carefully enclosed in one hand to expose the fins to be removed, and then the required fins were quickly excised. Marked smolts confined for a day or two in screen cages in gentle current showed no evidence of short-term marking mortality.

From 1950 to 1961 adipose and left ventral fins were removed from 174,509 smolts on the Northwest Miramichi, and adipose and right ventral fins from 95,262 Southwest Miramichi smolts (Table 1). As the same marking code was used at each site year after year, reading of scale samples from marked adults was necessary to identify the year of marking. In some instances scale samples were not taken at the time marked adult fish were examined; also, sometimes the samples comprised only regenerated scales. In such instances it was often possible to estimate the year of smolt marking from the weight or length data recorded at the time of adult capture. Though scale readings do not provide

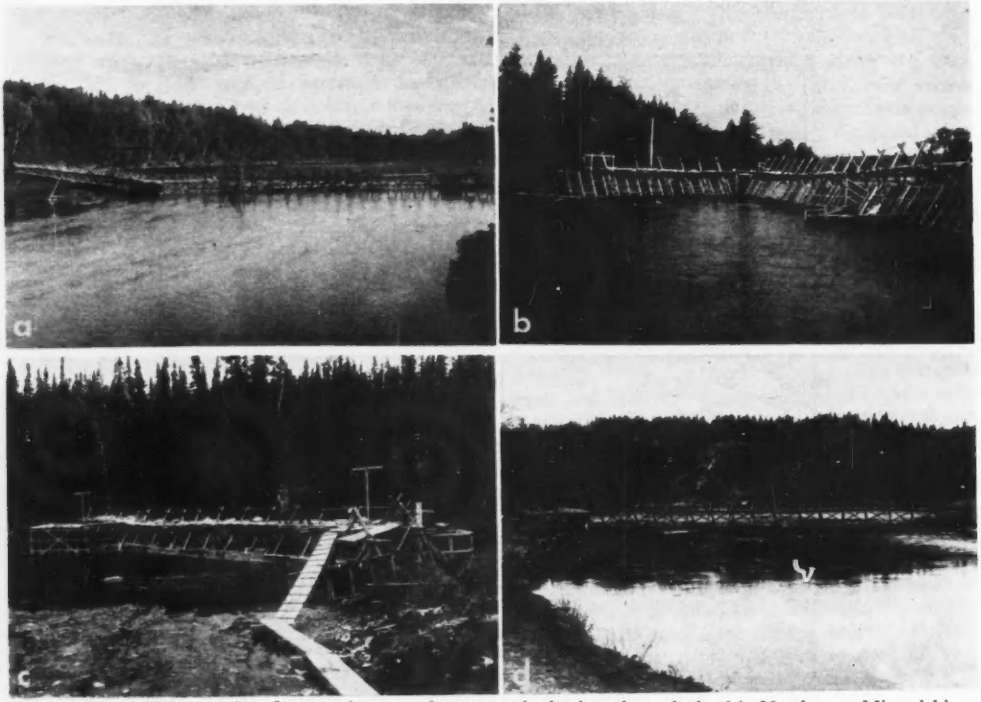

FIG. 3. Miramichi counting fences where smolts were obtained and marked: (*a*) Northwest Miramichi at Curventon, looking downstream; (*b*) Dungarvon River at Pineville; (*c*) Northwest Miramichi at Camp Adams; (*d*) Cains River at Shinnickburn.

a dependable estimate of the number of times a salmon has spawned if it has spawned twice or more (White and Medcof 1968), respawners comprise a very small percentage of the stock of fish in the sea or rivers. Blair (1935) found 12.8% previously spawned fish in a sample of 1383 salmon taken from Miramichi commercial gear, and in the present sampling the proportion was often under 5% (Allen and Lindsey MS 1967).

From 1951 to 1961 two trapnets were operated in the Miramichi estuary near Millbank from early May through June to capture samples of migrating smolts. The traps were patterned after the "double-end" box net used for smelt fishing in northeastern New Brunswick (McKenzie 1946). The traps were hauled daily and the finclipped and unmarked smolts were counted and recorded, taking care not to damage the fish, which were released. The total number captured often exceeded 35,000 per year and reached 76,000 in 1955. Sometimes 1000 finclipped smolts were taken per year and in 1955 the number exceeded 2200. This operation gave information on the condition of smolts a couple of weeks after they had been marked upriver and permitted a Petersen estimate (Ricker 1958) of total Miramichi smolt production.

Recaptures of Marked Adults

Various means were used to obtain as accurate and thorough information as possible on where finclipped adult salmon were recaptured. They included issuing instructions and annual progress reports to fisheries research and management personnel and to the public, and assigning technicians to check salmon catches at strategic places.

Wherever the Board trapped or otherwise captured adult salmon, they were examined for missing fins and marked fish were released after being measured and scale-sampled. The trapping sites were on the Pollett River (Elson 1962), the Little Codroy River (Murray 1968), and the Miramichi River. On the Miramichi, besides the two-way counting fences described above, an estuarial sampling trap for large salmon was operated annually, starting in 1952, at Millbank. It provided data on salmon runs before and after the public fishing season (mid-May to August 31 for commercial nets; mid-May to September 30 for angling, with minor exceptions). The Millbank trapnet resembled regular commercial salmon traps used nearby except that the netting was 3½-inch stretched mesh, instead of 5-inch, to retain grilse under 5 lb in weight. It was cleared of fish 6 or 7 days per week depending on the weather, whereas a 48-hr closure was enforced by regulation on public trapnets every weekend.

Persons responsible for checking salmon catches for marked fish in research gear and in catches made by the public were issued detailed instructions annually on the technique of examining fish, reporting the data, submitting scale samples, and paying rewards of $1 for each legitimate finclip. Doubtful instances were reported with accompanying fin scars and surrounding tissue to the St. Andrews station. Trained staff made frequent contact with the public in several fishing and packing areas, including many angling camps in the Miramichi watershed, packing houses handling Miramichi trapnet salmon, wharves near Escuminac, N.B., where most of the drift-net catches were landed, trapnet fisheries around Hall's Harbour, N.S., packing houses for locally caught and imported salmon at Saint John, N.B., and North Sydney, N.S., which was the chief landing point of Newfoundland commercial catches en route to Canadian and United States centres. Other special examinations of commercially caught salmon in Newfoundland, including Bonavista, Port Union, and St. Anthony, were described by Murray (1968).

Table 1. Numbers of Atlantic salmon smolts finclipped at marking traps, Miramichi River, N.B., 1950–61.

	Northwest Miramichi Adipose + left ventral		Southwest Miramichi Adipose + right ventral		Total	
Year	Curventon	Camp Adams	Dungarvon R.	Cains R.	Ad+LV	Ad+RV
1950	7969		253		7969	253
1951	33407		14966		33407	14966
1952	848		461		848	461
1953	25218		19996		25218	19996
1954	25660		20254		25660	20254
1955	25735		13057		25735	13057
1956	12733		9130		12733	9130
1957	779				779	
1958	17837	2916		8100	20753	8100
1959	18620	1145		6661	19765	6661
1960	34	1011		2384	1045	2384
1961	239	358			597	
Total	169079	5430	78117	17145	174509	95262

Bergeron (1959) outlined the examination of catches in the Province of Quebec.

Throughout the study a major problem was the evaluation of thoroughness of search for marked adults. As pointed out above, much effort was directed annually to advertising the marking program, instructing the fishing public and officials, and paying rewards for verified recaptures of finclipped fish. Because of the regular presence of an efficient Board staff in the Miramichi area, the returns there are considered to be particularly reliable. There was, however, difficulty in arousing enthusiastic participation in the program by most of the Miramichi angling camps; a few, however, did display effective interest. Commercial fishermen generally were interested in reporting recaptures, partly because the $1 rewards meant more to them than to most anglers. It is impossible to assign relative values to the effectiveness of the general search for marks over the large area involved, and the data are presented for what they are worth without attempting to apply correction factors that might only confuse the results. Adequate data are available, however, to assign a correction factor for commercial landings by Newfoundland fishermen. At the outset of the investigation it was decided, for Newfoundland commercial catches only, to accept finclip returns not from individual fishermen but only from observers stationed at strategic points where large quantities of salmon were repacked for trans-shipment. Initially all examinations were made at warehouses operated by several firms at North Sydney, N.S., and later the search was extended to Bonavista and St. Anthony in Newfoundland. In these instances the rewards for marked salmon were paid to the staff of the business firms who discovered them. Murray (MS 1966) presented detailed data and correction factors for the fractions of the total catch of grilse and larger salmon that were examined by observers each year. These were used to adjust the Newfoundland commercial recaptures in this paper.

Results

Commercial fisheries accounted for considerably more than half the total utilization of the finclipped fish — 62% for salmon of Northwest Miramichi origin and 74% for Southwest Miramichi salmon (Table 2). The bulk of the commercial recoveries was about equally divided among Newfoundland traps, Miramichi drift nets, and Miramichi trapnets; the remainder came from various commercial catches in the Maritime Provinces and Quebec. The percentages of grilse (1 sea-year fish) in commercial catches were low, 20% for Northwest and 17% for Southwest fish.

Anglers reported 10 and 7%, respectively, of all the recaptures of Northwest and Southwest fish. All these angling recoveries were made in freshwater sections of the Miramichi River. Large numbers of "nil" reports were received from fishery officers who examined catches in other streams in the Maritime Provinces and Newfoundland. Grilse comprised over 70% of angling recaptures of both Northwest and Southwest fish.

Spawning escapement accounted for 28% of the Northwest returns, of which 60% were grilse; and 19% of the Southwest returns, of which 85% were grilse.

For the total of 174,509 smolts marked on the Northwest Miramichi, the percentage minimum survival rate to fisheries and spawning escapement, not corrected for general thoroughness of search and other factors, was 2.7% (Table 2). For 78,117 Southwest smolts the corresponding value was 2.0% (Table 2).

Information on utilization of the 17,145 marked smolts from the Cains River is limited to the 295 reported in commercial fisheries (Table 3). None were reported by anglers, and no counting fence was operated on the Cains to record adult escapement. Correcting the Newfoundland commercial recaptures for partial examination at packing houses, as done for other tributaries of the Miramichi, raises the total Cains recaptures to 446 (2.6% utilization by commercial gear alone). Of these, 47% were recovered in Newfoundland, 51% in New Brunswick, and 2% in Nova Scotia nets and traps.

In the 11 years of operation of the smolt-sampling traps in the estuary, 1951–61, there were 6 years when the data were inadequate for estimates of smolt production either because only a small number of smolts were marked upriver or because few were captured in the estuary. Data for the 5 years in which they appeared to be reasonably adequate (Table 4) gave Peterson estimates of total Miramichi smolt production ranging from 0.8 to 2.6 million and averaging 1.6 million (standard error 0.4).

Discussion

Information on the utilization of Miramichi-produced salmon from this study, which involved extensive finclipping of smolts, is similar to that reported by Saunders (1969) on 5 years of tagging Northwest Miramichi smolts at the Curventon fence. Expressing Saunders' tag recaptures as percentages, gives, for the 1005 tagged smolts released, an average survival rate of the same order, namely 2.4%. Of the survivors in Saunders' study, 48% were taken in commercial fisheries and 20% by anglers, and 32% represented potential spawning escapement. The corresponding figures for Northwest finclipped smolts in my study, 62, 10, and 28%, entailed somewhat higher recovery by commercial fisheries and lower recovery by angling.

Both studies showed that many Miramichi-produced salmon were taken by commercial fisheries near Newfoundland as well as around the Maritime Provinces, and especially in the vicinity of the Miramichi. Neither study showed any angling recaptures outside the home river, confirming that Atlantic salmon do not tend to wander into freshwater sections of other than their natal river. The same is shown by the extremely small exchange of finclipped salmon between Northwest and Southwest counting fences (Table 3).

Saunders' results differ from mine also in showing

TABLE 2. Recaptures of grilse and larger salmon in commercial and angling fisheries and at counting fences on Northwest Miramichi River (174,509 descending smolts finclipped by removal of adipose and left ventral fins from 1950 to 1961) and on Southwest Miramichi (78,117 descending smolts finclipped by removal of adipose and right ventral fins from 1950 to 1956).

Utilization	Grilse	Larger salmon	Total	% grilse	% of total utilization
		Northwest Miramichi			
Commercial fisheries					
Newfoundland[a]	315(39)	607(206)	922(245)	34.2	19.5
Nova Scotia	13	25	38	34.2	0.8
New Brunswick					
Miramichi drift nets	13	853	866	1.5	18.3
Miramichi trapnets	240	823	1063	22.5	22.4
Other	10	49	59	17.0	1.2
Quebec	2	3	5	40.0	0.1
Total commercial	593	2360	2953	20.0	62.2
Angling fishery					
Miramichi R.	339	126	465	73.0	9.8
Escapement[b]	796(202)	531(52)	1327(254)	60.5	28.0
Total	1728	3017	4745	36.4	100.0
Minimum survival rate of smolts to fisheries and spawning escapement = $\frac{4745}{174509}$ = 2.7%.					
		Southwest Miramichi			
Commercial fisheries					
Newfoundland[a]	113(16)	328(130)	441(146)	25.6	27.6
Nova Scotia	1	4	5	20.0	0.3
New Brunswick					
Miramichi drift nets	1	330	331	0.3	20.7
Miramichi trapnets	77	300	377	20.4	23.6
Other	3	14	17	17.6	1.1
Quebec		7	7	0	0.4
Total commercial	195	983	1178	16.6	73.6
Angling fishery					
Miramichi R.	78	30	108	78.0	6.8
Escapement[b]	266(52)	47(15)	313(67)	85.0	19.6
Total	539	1060	1599	33.8	100.0
Minimum survival rate of smolts to fisheries and spawning escapement = $\frac{1599}{78117}$ = 2.0%.					

[a]Corrected for partial examination of catch, year by year, as estimated by Murray (1966); actual counts in parentheses.

[b]Corrected for finclipped fish angled above counting fence; angled fish in parentheses.

recaptures of tagged Northwest Miramichi fish in the commercial fishery near west Greenland. But this fishery only developed after 1960, to produce a significant catch of over 1500 metric tons by 1964 (Anonymous 1969), too late to contribute to the smolt finclipping study. The possibility of finding finclipped adult salmon was not advertized then, and in any case it is unlikely that they would have been identified in catches.

My unadjusted survival rates for smolts to adults correspond with the value of 2.3% reported by Murray (1968) for smolts finclipped on the Little Codroy River, Nfld. Although their contribution to commercial fisheries was 61%, similar to mine, only 4% contributed to anglers' catches and 35% represented escapement. Murray adjusted his survival values upwards to take into account effects of handling and marking smolts and other factors. These adjustments raised the estimated Little Codroy smolt survival rate to 9.4%. Whereas I might guess that the present 2.0–2.7% survival rate for Miramichi could be increased by two or

TABLE 3. Numbers of adult salmon recaptured annually, after finclipping as smolts on the Northwest and Southwest Miramichi rivers from 1955 to 19[illegible]ercial an

Area	Recaptured in	Marked in 1950 (7969)				1951 (33407)			1952 (848)			1953 (25218)			1954 (25660)				Marked on Northmichi 1955 (25[illegible]6 (12733)		
		1951	1952	1953	1954	1952	1953	1954	1953	1954	1955	1954	1955	1956	1955	1956	1957	1958	1956	1957	1958
																					Commercial
Newfoundland																					
1. Labrador coast		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–
2. E coast of Is.		–	–	–	–	–	75	–	–	2	–	3	20	–	9	12	–	–	8	5	19
3. S coast of Is.		–	–	–	–	–	–	–	–	–	–	–	2	–	2	5	–	–	1	9	–
4. W coast of Is.		–	–	–	–	–	1	–	1	–	–	–	–	–	–	3	–	–	–	–	–
5. Unclassified		–	–	–	–	–	32	–	–	–	–	–	–	–	1	–	–	–	–	–	–
Total																					
Nova Scotia																					
1. Northumberland St.		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
2. Cape Breton, E & S		–	–	–	–	–	1	–	–	1	–	–	–	–	–	1	–	–	–	–	–
3. Cape Breton, W		–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–
4. Mainland		–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–
5. Bay of Fundy		–	–	–	–	–	–	–	–	–	–	–	1	–	–	3	–	–	–	–	–
Total																					
New Brunswick																					
1. Escuminac and G. of St. Lawrence driftnets		–	46	–	–	–	104	1	–	6	–	3	67	1	–	88	2	–	5	116	69
2. Miramichi Estuary trapnets		–	24	–	–	12	172	1	9	17	–	16	29	–	29	64	2	–	24	106	94
3. NW Miramichi trapnets		–	1	–	–	2	7	–	–	–	–	3	2	1	9	7	–	–	13	20	6
4. SW Miramichi trapnets		–	1	–	–	–	3	–	–	–	–	–	–	1	–	4	–	–	3	5	2
5. Chaleur Bay trapnets		–	3	–	–	–	5	–	–	1	–	–	6	–	2	1	–	–	1	–	–
6. Northumberland St. trapnets		–	–	–	–	–	6	–	–	–	–	–	–	–	–	–	–	–	–	5	3
7. Bay of Fundy trapnets		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Total																					
Quebec		–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	1	–	–
Total																					
																					Rod
1. NW Miramichi above Curventon		–	3	–	–	67	23	–	11	6	–	33	2	–	36	7	–	–	28	4	6
2. NW Miramichi below Curventon		–	3	–	–	10	2	–	17	–	–	5	5	–	2	2	–	–	–	3	–
3. Sevogle		–	–	–	–	5	1	–	2	–	–	6	–	–	1	2	–	–	1	2	1
4. Little SW Miramichi		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
5. Renous		–	–	–	–	1	1	–	–	1	–	1	–	–	–	–	–	–	–	–	–
6. Dungarvon		–	–	–	–	4	–	–	–	1	–	–	–	–	1	–	–	–	–	1	–
7. Main SW Miramichi above Blackville		–	1	–	–	20	1	–	1	–	–	2	–	–	1	–	–	–	–	–	–
8. Main SW Miramichi below Blackville		–	–	–	–	–	–	–	1	–	–	–	–	–	–	1	–	–	–	–	–
Total																					
Grand totals, all fisheries																					
																					Trap and Sites
NW Miramichi Fence (Curventon)		36	29	1	2	225	140	4	14	15	2	151	87	–	145	52	–	2	69	65	55
Dungarvon Fence (Pineville)		–	–	–	–	3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Estuary (Millbank) — excluded from fishery and escapement data		–	1	–	–	–	7	–	–	–	1	12	10	–	7	20	1	–	21	12	6

three times by allowing for inadequacy of search for marks and for other factors, it appears to be impossible to improve on the 5–10% value estimated as reasonable some time ago (Kerswill 1958). This was supported by the summary by Elson (1957) of results of various published returns of Atlantic salmon to fisheries and counting fences, where the rate ranged from 2 to 17% and averaged 8% (1.5% standard error).

The ratio of grilse to larger salmon in the recaptures supports the possibility implied by Saunders' (1969) data that the ratio in the Miramichi stock has increased in recent years. In the total recaptures by commercial and sport fisheries of fish tagged by Saunders from 1960 to 1965, grilse outnumbered larger salmon by 2.2:1. In the following table, comparison of total recaptures of the three groups of finclipped Miramichi fish presented in this paper (1951–64) with Saunders' tagged salmon data (1961–68) indicates a change from 0.62:1 to over 2:1 in the ratio in the Northwest Miramichi:

55 to 19[illegible]ercial and angling fisheries in eastern Canadian provinces and at trapping and marking sites. Numerals in parentheses are numbers of smolts marked.

Northwest Miramichi (Ad + LV)																							Totals of age classes by sea-years				
55 (257)[illegible]6 (12733)			1957 (779)					1958 (20753)			1959 (19765)				1960 (1045)			1961 (597)							
1957	1958	1959	1958	1959	1960	1961	1962	1959	1960	1961	1960	1961	1962	1963	1961	1962	1963	1962	1963	1964	1	2	3	4	5
Commercial																										
–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	1	1	–	–	–
5	19	–	3	1	2	–	–	1	11	–	7	–	–	–	–	–	–	–	–	–	32	145	2	–	–
9	–	–	–	–	–	–	–	–	–	–	–	3	–	–	–	–	–	–	–	–	3	19	–	–	–
–	–	–	–	1	–	–	–	1	1	–	–	1	–	–	–	–	–	–	–	–	2	7	–	–	–
–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	32	–	–	–
																					39	204	2	–	–
–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–
–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	3	–	–	–
–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–
–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–
–	–	–	–	–	–	–	–	–	1	–	13	14	–	–	–	–	–	–	–	–	13	19	–	–	–
																					13	24	1	–	–
Angling																										
116	69	1	–	13	4	2	–	3	71	3	1	233	2	4	–	17	–	–	2	–	13	832	15	6	–
106	94	–	4	24	4	2	–	9	89	6	66	103	4	1	1	3	1	–	–	1	194	725	20	3	–
20	6	–	–	2	–	–	–	–	9	–	9	2	–	–	–	–	–	–	–	–	39	56	3	–	–
5	2	–	–	–	–	–	–	–	–	–	2	–	–	–	–	–	–	–	–	–	7	15	1	–	–
–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	3	16	–	–	–
5	3	–	–	–	–	–	–	–	4	–	2	14	–	–	–	–	–	–	–	–	2	32	–	–	–
–	–	–	–	–	–	–	–	–	1	–	2	–	–	–	1	–	–	2	–	–	5	1	–	–	–
																					263	1677	39	9	–
–	–	3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2	–	3	–	–
																					2	–	3	–	–
Any																										
8 4	6	–	2	–	–	–	–	12	1	–	2	–	–	–	1	–	–	1	–	–	202	52	–	–	–
– 3	–	–	–	10	1	–	1	18	13	–	15	7	10	–	1	2	–	–	–	–	71	47	11	–	1
1 2	1	–	–	1	–	–	–	2	1	–	13	–	–	–	–	–	–	–	–	–	30	8	–	–	–
– –	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
– –	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2	2	–	–	–
– 1	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	6	2	–	–	–
– –	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	25	2	–	–	–
– –	–	–	1	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	3	1	–	–	–
																					339	114	11	–	1
																					656	2019	56	9	1
Trapping Sites																										
59 65	55	5	5	6	3	–	–	174	10	10	130	67	2	–	7	7	–	–	1	1	998	534	45	4	–
– –	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	3	–	–	–	–
21 12	6	–	2	3	–	–	–	2	10	2	16	9	–	–	1	–	–	–	–	–	63	78	7	–	–

(*Continued*)

TABLE 3. Numbers of adult salmon recaptured annually, after finclipping as smolts on the Northwest and Southwest Miramichi rivers from to 196 numbers of smolts marked. — (*Concluded*)

Area	Marked in / Recaptured in	1950 (253) 1951	1952	1953	1951 (14966) 1952	1953	1954	1952 (461) 1953	1954	1955	1953 (19996) 1954	1955	1956	1954 (20254) 1955	1956	1957	19 (13057) 1957	1
		Marked on Southwest Miram Dungarvon smolts																
																	Comm Fisherie	
Newfoundland																		
1. Labrador coast		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
2. E coast of Is.		–	–	–	–	60	–	–	1	–	3	19	–	2	11	–	3	
3. S coast of Is.		–	–	–	–	–	–	–	–	–	–	4	–	–	–	–	5	
4. W coast of Is.		–	–	–	–	1	–	–	–	–	3	–	–	3	5	–	1	
5. Unclassified		–	–	–	–	13	–	–	–	–	–	1	–	–	–	–	–	
Nova Scotia																		
1. Northumberland St.		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
2. Cape Breton, E & S		–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	
3. Cape Breton, W		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
4. Mainland		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
5. Bay of Fundy		–	–	–	–	–	–	–	–	–	–	2	–	–	–	–	–	
New Brunswick																		
1. Escuminac and G. of St. Lawrence drift nets		–	1	–	–	82	–	–	5	–	–	61	1	–	62	2	67	
2. Miramichi Estuary trapnets		–	–	–	5	45	–	2	10	–	13	26	–	20	35	–	80	
3. NW Miramichi trapnets		–	–	–	–	2	–	–	–	–	–	–	–	1	2	–	2	
4. SW Miramichi trapnets		–	–	–	–	7	–	–	–	–	–	–	–	1	8	–	7	
5. Chaleur Bay trapnets		–	1	–	–	2	–	–	–	–	–	4	–	3	1	–	–	
6. Northumberland St. trapnets		–	–	–	–	2	–	–	1	–	–	–	–	–	–	–	1	
7. Bay of Fundy trapnets		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
Quebec		–	–	–	–	–	–	–	–	–	–	3	4	–	–	–	–	
Total																		
																	Ad Fishery	
1. NW Miramichi above Curventon		–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	
2. NW Miramichi below Curventon		–	–	–	–	–	–	2	–	–	–	–	–	–	–	–	–	
3. Sevogle		–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	
4. Little SW Miramichi		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
5. Renous		–	–	–	2	1	–	2	5	–	–	–	–	1	–	–	1	
6. Dungarvon		–	–	–	11	1	–	12	5	–	11	6	–	12	–	–	–	
7. Main SW Miramichi above Blackville		–	1	–	13	1	–	2	–	–	–	–	–	–	2	–	–	
8. Main SW Miramichi below Blackville		–	–	–	1	–	–	–	2	–	–	–	–	–	1	–	–	
Total																		
Grand totals, all fisheries																		
																	Trappi Marking	
Dungarvon Fence (Pineville)		–	–	–	91	36	1	–	2	1	137	13	–	72	9	–	(No fen	
NW Miramichi Fence (Curventon)		–	–	–	1	–	–	–	–	–	1	1	–	3	1	–	–	
Estuary (Millbank) — excluded from fishery and escapement data		–	–	–	1	10	–	–	1	1	8	9	–	18	5	–	2	1

from 1 to 1961, in commercial and angling fisheries in eastern Canadian provinces and at trapping and marking sites. Numerals in parentheses are

outhwest Miramichi (Ad + RV)

									Cains smolts (not included in Table 2)													
	1 (13057)		1956 (9130)			Totals of age classes by sea-years			1958 (8100)			1959 (6661)			1960 (2384)				Totals of age classes by sea-years			
7 1956	1957	1958	1957	1958	1959	1	2	3	1959	1960	1961	1960	1961	1962	1961	1962	1963	1964	1	2	3	4
Commercial Fisheries																						
	–	–	–	–	–	–	–	–	–	–	–	–	2	–	–	–	–	–	–	2	–	–
5	3	–	–	5	–	10	99	–	–	28	–	9	1	–	–	–	–	–	9	29	–	–
	5	–	–	–	–	–	9	–	–	5	–	–	3	–	–	–	–	–	–	8	–	–
	1	–	–	1	–	6	8	–	–	8	–	3	–	–	–	–	–	–	3	8	–	–
	–	–	–	–	–		14	–	–	–	–	–	–	–	1	–	–	–	1	–	–	–
					Total	16	130	–										Total	13	47	–	–
	–	–	1	1	–	1	1	–	–	–	–	1	–	–	–	–	–	–	1	–	–	–
	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	–	–	–	–	–	–	2	–	2	–	–	1	3	–	–	–	–	–	3	3	–	–
					Total	1	4	–										Total	4	3	–	–
2	67	2	1	47	–	1	325	5	–	59	8	–	100	2	2	16	–	–	2	175	10	–
12	80	2	17	67	–	69	263	2	3	15	1	3	12	–	–	–	–	–	6	27	1	–
9	2	–	–	3	–	3	9	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
3	7	2	1	2	–	5	24	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–
4	–	–	–	1	–	3	9	–	–	–	–	–	1	–	–	–	–	–	–	1	–	–
1	1	1	–	–	–	–	4	1	–	2	–	1	–	1	–	–	–	–	1	2	1	–
	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	1	–	–	1	1	–	–
					Total	81	634	10										Total	10	206	12	–
	–	–	–	–	–	–	3	4	–	–	–	–	–	–	–	–	–	–	–	–	–	–
						–	3	4											–	–	–	–
Angling Fishery																						
	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	–	–	–	–	–	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
1	1	–	–	–	–	6	7	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	–	–	5	3	–	52	15	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	–	–	–	1	–	15	5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	–	–	–	–	–	1	3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
						78	30	–														
						176	801	14											27	256	12	–
Trapping Marking Sites																						
	(No fence after 1956)					318	60	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	–	–	–	1	–	6	3	–	1	–	–	1	–	–	1	–	–	–	3	–	–	–
	2	1	2	2	–	40	29	1	–	2	3	5	2	–	–	–	–	1	–	4	3	1

TABLE 4. Estimates of total annual smolt production of Miramichi River system, on basis of catches of finclipped smolts in estuarial sampling traps operated at Millbank, N.B.

Year	No. marked at upriver traps (Ad+LV; Ad+RV) T	Total no. captured in estuarial traps n	No. marked among estuarial catch t	Estimated total Miramichi smolt production $N = \frac{Tn}{t}$ millions
1953	45214	17615	989	0.8
1954	45914	35592	1068	1.5
1955	38792	76818	2265	1.3
1956	21863	58215	679	1.9
1958	28853	69855	776	2.6
				Avg 1.6±0.4 (SE)

Marked fish	Total recaptures: Grilse	Total recaptures: Larger salmon	Ratio
Northwest Miramichi, finclipped	1720	2753	0.62:1
Dungarvon, finclipped	540	910	0.59:1
Cains, finclipped	30	276	0.11:1
Northwest Miramichi, tagged	690	315	2.2:1

Records of ascending fish at counting fences in fresh water, for example Fig. 1, and also those of anglers' catches give a biased picture of the ratio of grilse to larger salmon in the total salmon production of a river, because the commercial fishery in salt water has already removed many larger salmon from the run. Total recoveries for the two fisheries give a truer representation of the ratio. The changing ratio has important management implications. For example, it suggests a need to consider further restrictions on commercial salmon fisheries to allow the escape of more larger salmon for angling and reproduction. But such changes in management policy should be based on new research, especially on the genetic effects of differential rates of utilization of grilse and larger salmon.

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A Comparison of a Simple Corer and an Ekman Grab for Sampling Shallow-Water Benthos

C. G. PATERSON[1] AND C. H. FERNANDO

Department of Biology
University of Waterloo, Waterloo, Ont.

PATERSON, C. G., AND C. H. FERNANDO. 1971. A comparison of a simple corer and an Ekman grab for sampling shallow-water benthos. J. Fish. Res. Bd. Canada 28: 365–368.

Estimates of the density of freshwater benthic invertebrates in shallow lentic habitats were found to be about 50% higher when samples were taken with a simple corer than when samples were taken with an Ekman grab. The use of the corer also decreased the volume of sediment collected and reduced the tediousness of examining large samples.

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PREVIOUS investigations (Kajak 1963) have shown that macrobenthic invertebrates are often neither randomly nor uniformly distributed in shallow-water lentic habitats. The possible occurrence of contagious distributional patterns requires the collection of several benthic samples from each sampling station in order that a sample mean with desired limits of accuracy may be obtained. The actual number of required samples is dependent on the abundance of the fauna and the selected level of accuracy. However, the necessity of collecting a number of samples from each sampling site, in addition to the increased number of sites needed to adequately sample a heterogeneous habitat, often results in an effort requirement beyond the feasibility of many studies. The sorting of benthic invertebrates from the associated substrate material, particularly in shallow waters where the high proportion of coarse organic and inorganic material prevents easy concentration of the fauna by sieving or flotation, is tedious and very time consuming. For these reasons it would be desirable to use a sampler that lifts small volumes of substrate material. In shallow waters the additional effort involved in collecting more small samples as opposed to fewer large ones would be of little consequence in comparison with the time saved in sorting a smaller total volume of material.

The properties of a simple core sampler were compared with those of a conventional Ekman grab in an attempt to reduce the effort required to adequately sample the benthos of shallow water habitats. Corers of various designs and cross-sectional areas have been employed by several workers (e.g., Mundie 1957; Lenz 1955) in the study of benthic communities. Comparisons of their sampling efficiencies with those of other sampling devices are largely lacking. Kajak (1963) has compared the mean population density of chironomid larvae as determined from samples taken with a 225-cm² Ekman grab and with corers of 10 and 5 cm². His results show that the average density per 10 cm² as calculated from Ekman grab samples is substantially greater than that obtained with either corer. The 10-cm² corer gave a higher average density than the 5-cm² one. Kajak concludes that the lower estimates result from the high perimeter-to-surface area ratio of the corers. Presumably, a coring device that is not fully open generates a pressure wave in front of it on descent and would disperse the fauna before penetrating into the substrate.

Prejs (1969), using corers of cross-sectional areas 10 cm² and 45 cm², found that neither was adequate for sampling the highly mobile larva of *Chaoborus flavicans*, as much higher average densities were obtained with a 225-cm² Ekman grab. However, similar densities of oligochaetes were obtained with a 10-cm² corer, a 45-cm² corer, and a 225-cm² Ekman grab. The average abundance of chironomid larvae as determined by the 45-cm² corer and the 225-cm² grab were also similar. Prejs concluded that the benthic fauna could be adequately estimated with five 45-cm² core samples or three 225-cm² Ekman grab samples. The use of the 45-cm² corer decreased the volume of sediment needed to be examined by 67%, provided equal penetration is assumed.

Brinkhurst et al. (1969) have recently shown that the KB corer and the Ekman grab gave similar population densities of oligochaetes and other benthic fauna.

[1]Present address: Department of Biology, Mount Allison University, Sackville, N.B.

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Methods

To test the efficiency of a small sampler for sampling shallow-water benthos, a simple corer was designed with a cross-sectional area of 25.6 cm² and a tube length of 20 cm. This length was sufficient for inshore sediments as cores longer than 12 cm were rarely obtained. The tube was made of aluminum tubing 2 mm thick with an internal diameter of 5.7 cm. The leading edge was sharpened on the outside to facilitate penetration. From the top of the core tube two aluminum straps extended upwards for 20 cm to a narrow crossbar. This was fitted with a 3/8″ bolt for attachment to a nut welded into the end of a 2-m length of ½″ diameter iron pipe. A ferrule on the other end of the pipe allowed easy attachment of a second 2-m section. The upper opening of the tube was fitted with a 0.7 mm thick, hinged brass flap. The slightest water pressure on descent caused the flap to remain fully open; during ascent the flap would close tightly and retain the core. The development of a pressure wave was minimized by placing the attachment rod 20 cm from the tube, and by having the tube completely open on descent. A later model of the corer with a tube length of 60 cm was used successfully in soft sediments of lakes. The addition of a rubber ring to the underside of the brass flap provided a tight seal and allowed the corer to be lifted from the water with little danger of the sample being lost.

The Ekman grab used in this study was the standard 6-inch (231-cm²) cubical type with an area 9.05 times as great as that of the corer. It was modified for attachment to the jointed iron rod used on the corer. By attaching the corer and grab to a rigid pipe the positioning of the sampler could be controlled and even settling was assured.

Three sample series (A–C) were collected from 2-m² areas at depths of 0.8–2.0 m in Laurel Creek Reservoir (Paterson and Fernando 1969) and one from Lake on the Mountain, a marl lake near Glenora, Ont. (D). Samples were collected in calm weather from a boat positioned by heavy bow and stern anchors. Substrate materials were washed into polyethylene bags. In the laboratory the excess water was decanted off through a 0.3-mm mesh nylon net. All substrate material was preserved in 5% formalin. Before examination each sample was floated four times in a near-saturated solution of magnesium sulfate. Few organisms were recovered after the second flotation and none were found when the sediment in selected samples was examined microscopically after the fourth flotation. Benthic macroinvertebrates were separated from the organic debris by examining small portions at a time in a counting tray under 12.5 magnifications.

Results and Discussion

Table 1 shows the mean number of invertebrates collected with the two sampling devices and the number expected if the corer was used to sample an area equal to that sampled by the grab. The core sampler captured greater numbers of organisms per unit area than did the Ekman grab. As shown in the last column of Table 1 the average density calculated from the core samples was usually between 107 and 233% of that found with the Ekman grab. The only exception involved the oligochaetes in series A. On the basis of the averages of the four series, the total fauna (147.8%), chironomids (150.3%), and the oligochaetes (174.7%) are more effectively sampled by the corer than by an Ekman grab.

The greater estimates resulting from the use of the corer were unexpected. The corer penetrates deeper into the substrate and captures organisms living at greater depths than those reached with an Ekman grab. However, the percentage of the fauna commonly found below a depth of about 5 cm (Mundie 1957) is inadequate to explain the differences in the estimates we obtained. Samples collected from Laurel Creek Reservoir after winter drainage revealed very few benthic invertebrates below a depth of 6 cm (Paterson and Fernando 1969). A potential source of error could be the flushing out of water and substrate materials through the jaws of the Ekman grab when it is hauled upwards. However, crude experiments performed by injecting 100 cc of 1% Rhodamine B dye into a closed grab in about 1 m of water revealed that no appreciable interchange between the water in the grab and the surrounding medium occurred.

The smaller population estimates obtained with the Ekman grab may be partly explained by its basic design, which prevents the upper hinged flaps from opening completely on descent. This would generate a pressure wave in front of the grab and disperse some of the fauna before the grab penetrates the substrate. The Ekman grab also has the disadvantage of having jaws that are closed after it has settled into the substrate. In the harder sediments common in shallow inshore waters, the shape of the "bite" taken by the grab is such that the substrate near the leading edges of the jaws is sampled only to a shallow depth. The downward thrust of the jaws into a hard sediment can cause the body of the grab to be forced upwards, effectually reducing the depth of penetration. This is substantiated by the observation that the grab used in this study covered an area 9.05 times greater than that of the corer, but only collected about six times as much substrate material.

The only disadvantage of the corer seemed to be the capture of rare species. An equal number of Ekman grab samples captured more species because of the larger area sampled. However, as shown by Lloyd (1967), the population densities of rare species, particularly when strongly aggregat-

TABLE 1. The average density of benthic macroinvertebrates as determined with an Ekman grab and a corer and the relation between the two samplers when prorated to equal areas. The relative efficiency of the corer as a percentage of the efficiency of the grab is given in the last column.

	I Ekman grab 231 cm²	II Corer 25.6 cm²	III Corer 231 cm²	III/I %
		Series A		
No. samples	9	6		
No. species	19	14		
Total fauna	197.9±29.0[a]	26.7±4.0	241.6	122.1
Chironomidae	183.4±26.7	24.8±3.9	224.4	122.4
Oligochaeta	5.4±1.4	0.5±0.2	4.5	83.7
		Series B		
No. samples	9	8		
No. species	18	11		
Total fauna	82.2±12.9	15.3±1.5	138.0	167.9
Chironomidae	16.2±3.6	4.1±1.0	37.4	230.9
Oligochaeta	38.2±5.6	6.4±1.3	57.7	151.0
		Series C		
No. samples	9	9		
No. species	24	14		
Total fauna	105.8±11.3	20.9±2.3	189.1	178.7
Chironomidae	60.1±7.2	9.3±1.3	84.4	140.4
Oligochaeta	32.6±4.4	8.3±2.2	75.4	231.3
		Series D		
No. samples	9	9		
No. species	35	20		
Total fauna	142.6±9.2	19.3±2.4	174.7	122.5
Chironomidae	87.9±7.0	10.3±1.3	94.5	107.5
Oligochaeta	6.9±2.0	1.8±0.8	16.1	233.3

[a]One standard error.

ed, cannot be accurately determined even with large numbers of samples.

The edge effect of the 25.6-cm² corer did not produce the great sampling inaccuracies found for the much smaller corers used by Kajak (1963). The 25.6-cm² corer was completely open, which probably prevented the establishment of an appreciable pressure wave on descent. Because of the difference in the shape of the "bite" taken by the two samplers, the volume of sediment collected by the corer was about ⅛ that collected by the Ekman grab. Several core samples, therefore, can be used to determine the population densities of abundant species with less effort than is currently expended in the common, although inaccurate, procedure of collecting single Ekman grab samples from each site. As it would be feasible to mount several core tubes on one sampling device the time required for obtaining the samples could be reduced. Moreover, small samples can be more efficiently sorted than large ones. Zicsi (1962) has shown that the density of earthworms, as determined from hand sorting of various sized samples, decreased as the sample size increased. This is related to the increased tediousness, and consequent inefficiency, of sorting one large sample as opposed to several discrete small samples.

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X-Ray Spectrometric and Multivariate Analysis of Sockeye Salmon (*Oncorhynchus nerka*) from Different Geographic Regions

J. R. CALAPRICE

Fisheries Research Board of Canada
Biological Station, Nanaimo, B.C.

CALAPRICE, J. R. 1971. X-ray spectrometric and multivariate analysis of sockeye salmon (*Oncorhynchus nerka*) from different geographic regions. J. Fish. Res. Bd. Canada 28: 369–377.

The chemical composition of samples of fry and adult sockeye salmon (*Oncorhynchus nerka*) collected in different areas was characterized by X-ray fluorescence spectrometry. Multivariate analysis of the spectra indicate that fish taken from an area possess recognizably distinct chemical compositions. Discriminant functions computed using spectral data were used to classify unknowns successfully. The relative similarity of groups characterized by fluorescence spectrometry is shown by canonical analysis and the nature of the chemical differences is discussed. The results indicate that these aquatic organisms possess a chemoprint, a form of natural tag.

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THE pattern of movement of organisms may be considered to be one of the most important factors in the population biology of a species. This is one area in which the disciplines of ethology, ecology, and genetics overlap considerably. Much of our present knowledge about the detailed movement of salmon necessary for the management of species originates from studies in which organisms were captured, marked, released, and then recaptured (Foerster 1968). In marking organisms in field studies, one usually encounters difficulties in obtaining random samples of the organisms to be marked, finding an appropriate marking technique that can be easily applied without affecting the behavior of the organism, and recovering and recognizing tagged organisms. Ideally, one would like a form of natural tag.

This study examines the possibility that sockeye salmon (*Oncorhynchus nerka*) living in different environments acquire a chemical composition that is quantifiable, and that may be used as a basis for determining the geographic origin of groups of organisms. In this study samples of sockeye salmon fry and adults were collected from different freshwater habitats where convenient, and the chemical composition was characterized by X-ray fluorescence spectrometry. The results were then used as a basis for discriminating among specimens from different geographic areas using multivariate statistical techniques.

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Materials and Methods

ORIGIN OF SAMPLES

Sockeye salmon fry were captured in streams while migrating to lakes. Adult sockeye salmon were also collected from streams. These were fish that migrated to sea, returned, and spawned, presumably in their parent streams. The fry were preserved whole in formalin solution diluted to 10% with distilled water. Only the tail section of the adults was taken. Samples from the Fraser River System, B.C., were made available by the International Pacific Salmon Fisheries Commission. The location of the collection sites for each sample is illustrated in Fig. 1.

METHOD OF CHEMICAL ANALYSIS

Individual fish from each area were numbered and their chemical composition characterized by X-ray fluorescence spectrometry. This method of chemical analysis (Bowman et al. 1966) has been used recently to detect introduced chemical elements foreign to fish tissue as a marking technique (Calaprice and Calaprice 1970). In this present study each organism was irradiated with a gamma ray source, 25 mCi of Americium-241, which results in an X-ray fluorescence pattern that is characteristic of the kind and concentration of each element present. The X-ray system, a lithium drifted silicon detector, pulse height analyzer, and related electronics, was described in detail previously (Calaprice and Calaprice 1970). Differences in the system used in this study relate only to gain settings, a more intense radiation source with a different geometry, and an automatic sample changer. The equipment was adjusted so that X-rays with energies between 8.4 and

47.1 keV would be detected. There are 72 different chemical elements (atomic numbers 29–100) that produce fluorescent X-rays within this energy range and some elements may produce as many as 13 different energetic forms.

Individual fish were coded, dried for 24 hr at 102–105 C, and mounted on a circular sample changer such that the tail section, the area between the dorsal and caudal fins, was exposed to radiation. The ordering of samples on the sample changer, which holds 40 samples, was determined by using random numbers. All chemical analyses were carried out in the evening, during quiet hours. Each sample was irradiated for 1400 sec and the accumulated counts in each of the 100 channels of the pulse height analyzer were simultaneously printed on paper and coded on paper tape. At the conclusion of each analysis the data on each individual fish was in the form of a 100-word array, with each word, except the first, containing the number of X-rays for a given energy range. The counting time, a constant, was recorded in the first channel. This array coded on paper tape was then read into the computer, I.B.M. cards were produced, and the number of counts in each channel were divided by the total number of counts in the background peak that occurred at channel 24, the 24th word in the array. This transformation converted the data from a form that is a function of the absolute concentration of the elements to a form approximating the relative concentration of each element in the sample.

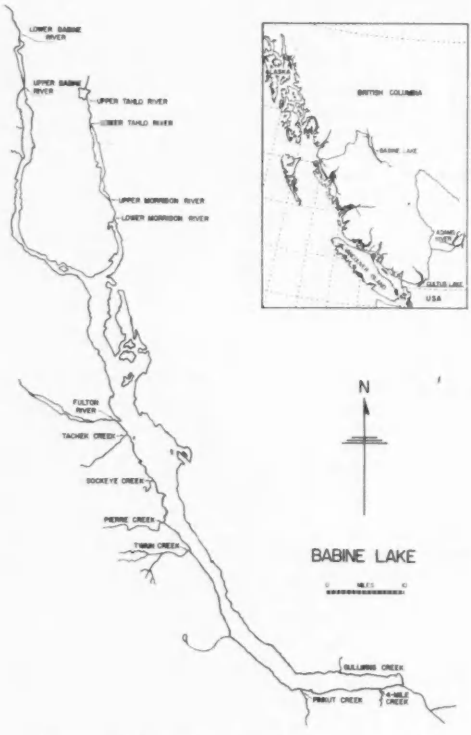

FIG. 1. Sites where collections were made.

STATISTICAL ANALYSIS

Multivariate statistical techniques provide a useful means of dealing with sets of observations on the same individual (Seal 1964). In this study the set of observations or variables characterizing the individual is its X-ray spectrum, an array of 100 six-digit words. To use the 1130 computer for the bulk of the analyses, the array was reduced to 40 by choosing alternate channels starting with channel 11.

The structure of the data in this study consists of measurements of individuals taken from each of a number of areas, with each individual characterized by a set of 40 scores. Multivariate techniques were used to determine whether or not differences in the spectra, i.e., chemical constituents, of the groups were statistically significant. Discriminant functions were computed to determine if individuals could be assigned to their area of origin. Canonical analysis was used to compare and to study the nature of the chemical differences among groups.

The discriminant function analyses were performed at Nanaimo, B.C., using programs described by Cooley and Lohnes (1962) and modified for the I.B.M. 1130 computer by Pienaar and Thomson (MS 1969) and Thomson and Calaprice (MS 1970). The stepwise multiple regression analysis and canonical analysis were completed at the University of British Columbia (I.B.M. 360/67) using a BIOMED series program BMD 07M (Dixon 1965).

Results

SOCKEYE FRY

The results of the multivariate analysis of fry samples demonstrates that the groups of fish from different regions have different chemical compositions. The statistical significance associated with the null hypothesis that group mean vectors are equal is less than 0.0001 (Table 1A).

With 40 variables and seven groups, a total of six discriminant functions may be computed. The eigenvalues or latent roots, λ, associated with each of the functions are given in Table 1B. Among the six functions computed, only the first three are statistically significant. The P values associated with the computed χ^2 values are less than or equal to 0.005. The first function accounted for 30.4% of the discrimination (percent of trace) and the first three functions combined accounted for 73.2% of the variance (cumulative percent of trace).

The individual fish that formed the samples from each area were then classified using the derived functions and the 40 values generated by the chemical

analysis (Table 1C). These results demonstrate that 77.4% of the fish were correctly reassigned to their area of origin. The pattern of misclassification provides some indication of the similarity among groups, i.e., the sample from Four Mile Creek appears to be quite distinct.

The above discriminant analysis utilized all 40 variables. A second analysis, a stepwise discriminant analysis, was conducted using the same data. With this analysis it is possible to test the differences between pairs of samples (Table 2). These results indicate that each of the samples compared are significantly different from each other with the exception of the Upper Babine River group. These fish do not appear to be distinguishable from either the Lower Babine River group or the Pinkut Creek group.

Sockeye Adults

As in the analysis of the fry, the sockeye adult samples are chemically distinct. The probability of observing such differences in group mean vectors by chance alone is less than 0.001 (Table 3A).

With 40 variables and 13 groups being compared, 12 discriminant functions were computed, the first four of which are significant and account for 73.3% of the variance.

The number of adults correctly assigned to their area of origin is 73.1% (Table 3C). As with the fry the pattern of misclassification gives some indication of the degree of similarity among groups. This will be studied in greater detail using canonical analysis.

Comparisons between all possible pairs of adult samples are shown in Table 4. The underscored contrasts in the half matrix are considered statistically distinct.

Classification of Unknowns

The adult sockeye samples used in the preceding analysis were divided into three groups, those from Adams River, Cultus Lake, and Babine Lake.

Table 1. Analysis of fry (Babine Lake system).

A. Test of hypothesis that group mean vectors are the same:

Wilks $\lambda = 0.0687$ $F(240,627) = 1.487$ $P = <.0001$

B. Eigenvalues and tests of significance for each function:

	% of trace	Cumulative % of trace	χ^2	df	P
$\lambda_1 = 1.0812$	30.44	30.4	92.5	45	<.005**
$\lambda_2 = .8424$	23.72	54.2	77.5	43	<.005**
$\lambda_3 = .6766$	19.05	73.2	65.4	41	.005**
$\lambda_4 = .4153$	11.69	84.9	43.9	39	.25 NS
$\lambda_5 = .3575$	10.06	95.0	38.6	37	.50 NS
$\lambda_6 = .1782$	0.50	100.0	20.6	35	.98 NS

C. Reclassification of individuals:

	Location assigned from functions							
Area of origin	Four Mile Cr.	Pinkut Cr.	Gullwing Cr.	Pierre Cr.	L. Babine	U. Babine	Fulton R.	Σ
Four Mile Cr.	15	1	–	–	–	–	–	16
Pinkut Cr.	–	19	2	1	1	1	–	24
Gullwing Cr.	1	2	17	–	2	1	1	24
Pierre Cr.	–	2	–	11	1	–	–	14
L. Babine R.	–	1	1	1	19	1	1	24
U. Babine R.	–	1	2	1	3	15	2	24
Fulton R.	–	–	1	1	–	2	20	24

% correct reclassification = 77.4

TABLE 2. Comparison of individual samples (fry). Probability that sample group mean vectors were taken from the same population ($\mu_i = \mu_j$).

	j					
i	Four Mile Cr.	Pinkut Cr.	Gullwing Cr.	Pierre Cr.	L. Babine R.	U. Babine R.
Pinkut Cr.	.0001					
Gullwing Cr.	.0366	.0005				
Pierre Cr.	.0016	.0001	.0004			
L. Babine R.	.0001	.0126	.0027	.0541		
U. Babine R.	.0000	.2694	.0002	.0088	.5664	
Fulton R.	.0001	.0013	.0001	.0008	.0010	.0001

TABLE 3. Analysis of sockeye salmon adult tails.

A. Test of hypothesis that group mean vectors are the same:

Wilks $\lambda = 0.1605$ $F(480,3968) = 1.4239$ $P = <.001$

B. Eigenvalues and tests of significance for each function:

	% of trace	Cumulative % of trace	χ^2	df	*P*
$\lambda_1 = 0.9131$	41.71	41.71	238.0	51	<0.005**
$\lambda_2 = 0.2821$	12.88	54.60	91.0	49	<0.005**
$\lambda_3 = 0.2304$	10.52	65.12	76.0	47	<0.005**
$\lambda_4 = 0.1795$	8.20	73.33	60.5	45	0.05*
$\lambda_5 = 0.1298$	5.93	79.26	44.7	43	0.50 NS
$\lambda_6 = 0.1155$	5.27	84.54	40.6	41	0.50 NS
$\lambda_7 = 0.0950$	4.34	88.88	40.0	39	0.50 NS
$\lambda_8 = 0.0698$	3.18	92.07	39.1	37	0.50 NS
$\lambda_9 = 0.0567$	2.59	94.66	38.7	35	0.75 NS
$\lambda_{10} = 0.0517$	2.36	97.02	38.4	33	0.75 NS
$\lambda_{11} = 0.0361$	1.65	98.67	38.0	31	0.75 NS
$\lambda_{12} = 0.0289$	1.32	99.99	37.7	29	0.75 NS

C. Classification based on probability values:

		1	2	3	4	5	6	7	8	9	10	11	12	13
1	Fulton R.	22	1	–	–	2	–	–	–	1	1	1	1	1
2	Tachek Cr.	2	20	2	–	–	–	–	2	2	–	2	–	–
3	Sockeye Cr.	–	2	16	1	–	2	1	1	–	2	–	1	4
4	Pierre Cr.	–	1	2	24	1	1	1	–	–	–	–	–	–
5	L. Babine R.	3	1	1	1	18	–	2	2	–	–	–	1	1
6	Pinkut Cr.	–	–	–	1	–	25	–	1	–	1	–	2	–
7	U. Babine R.	1	2	–	–	1	3	20	–	1	–	–	–	1
8	U. Tahlo R.	2	–	1	–	–	–	–	22	–	–	–	1	1
9	Adams R.	1	2	1	–	1	–	–	–	20	–	–	–	–
10	Cultus L.	–	–	2	–	–	1	1	–	–	44	1	1	–
11	L. Tahlo R.	1	–	–	1	–	–	–	1	–	–	19	1	–
12	U. Morrison R.	3	–	–	1	–	–	–	1	1	–	–	17	3
13	L. Morrison R.	1	–	2	1	1	1	–	1	1	1	1	–	20

= 287/393 = 73.1%

Combined, there were 333 samples from the Babine Lake and, as before, 25 samples from the Adams River and 50 samples from Cultus Lake. An equal number of individuals were selected from each of the different Babine groups that were originally pooled to give the 333 samples. These were selected using random numbers, and were withdrawn from the main body of data. Discriminant functions were derived using the remaining individuals from the three groups. These functions were then used to classify the unknowns, the selected subsample. All 40 variables were used in the analysis. The procedure was repeated, each time using a larger number of samples drawn from the Babine system and treated as unknowns. The relation between the size of sample used to derive the functions and the proportion of unknowns classified correctly is illustrated in Fig. 2. Increasing the sample size used in deriving functions has the obvious effect of reducing the number of misclassifications.

Canonical Analysis and the Nature of Chemical Differences

Canonical analysis provides a technique for comparing groups that are characterized by a number of different scores. It is a useful method for illustrating the relative closeness of the samples as determined from means of the X-ray counts in each channel. This we know to be related to the chemical composition of the different samples. The method of computation is described by Anderson (1958) and Seal (1964). The mean vectors of the seven groups of fry and 13 groups of adult sockeye are expressed in canonical form in Fig. 3 and 4.

Four Mile and Gullwing Creek fry samples (Fig. 3) are closely associated and these are from the south end of the lake. Lower and Upper Babine River are associated and these are from the north end of the lake. It would appear that samples from adjacent geographic areas are more similar chemically. The associations in Fig. 4 based upon the chemical composition of the adult samples support this conclusion. The Babine Lake samples are at the left of the graph, the Cultus Lake sample is located to the right, and the Adams River sample is intermediate. Within the Babine Lake group, the samples collected from the northern area of the lake occupy the uppermost section of the graph of canonical variates.

The nature of the chemical differences that account for the separation of groups of fish is difficult to ascertain for reasons to be discussed. It is possible, however, to examine the spectrum and readily determine which sections or variables provide for the greater degree of separation. Discriminant analyses were performed at different stages (stepwise), each time choosing the new variable that adds most significantly to discrimination. The results of the analyses are given in Table 5. The analyses demonstrate that in the comparison of groups of sockeye fry, 11 steps were performed and only these caused a sufficient reduction in variance to be used in the analyses. Of the variables used, variable 22 resulted in the greatest decrease in variance and variable 10 caused the least significant change. All other variables

TABLE 4. Comparison of individual samples (adult). Probability that sample group mean vectors were taken from the same population ($\mu_i = \mu_j$).

	Fulton R.	Tachek Cr.	Sockeye Cr.	Pierre Cr.	L. Babine R.	Pinkut Cr.	U. Babine R.	U. Tahlo R.	Adams R.	Cultus L.	L. Tahlo R.	U. Morrison R.
Tachek Cr.	.929											
Sockeye Cr.	.674	.635										
Pierre Cr.	.500	.083	.166									
L. Babine R.	.032	.003	.177	.006								
Pinkut Cr.	.664	.270	.058	.575	.001							
U. Babine R.	.028	.001	.090	.054	.096	.082						
U. Tahlo R.	.067	.002	.018	.033	.651	.021	.033					
Adams R.	.010	.001	.010	.000	.055	.000	.001	.125				
Cultus L.	.000	.000	.000	.000	.000	.000	.000	.000	.000			
L. Tahlo R.	.051	.024	.353	.001	.207	.003	.134	.024	.282	.000		
U. Morrison R.	.464	.051	.179	.679	.002	.505	.159	.007	.000	.000	.016	
L. Morrison R.	.028	.005	.356	.006	.164	.002	.161	.003	.002	.000	.406	.145

were excluded from the analyses because the probability for inclusion was greater than 0.1. The probability for inclusion of each variable, the U statistic, approximate F, and associated probability that the group mean vectors are distinct along with the X-ray energy associated with that variable is given in Table 5.

In the analyses of adults, nine variables were selected, only four of which were the same as those used in the comparison of fry samples (Table 5). Apparently the differences in chemical constituents that allow separation of fry samples are not the same as those useful for separating adults.

Discussion and Conclusions

Young salmonids when irradiated as controls in an earlier tagging study exhibited a background of X-ray fluorescence that suggested the presence of a number of different elements in trace quantities (Calaprice and Calaprice 1970). The fact that this pattern was variable led to the hypothesis that organisms may possess a form of "chemoprint." To test the hypothesis, samples of salmon from different geographic areas were captured and irradiated. In an earlier study samples of coho salmon (*Oncorhynchus kisutch*) smolts and sockeye salmon smolts were collected and irradiated. The analysis indicated that samples of coho from different regions and samples of sockeye smolts had recognizable differences in the X-ray spectra. Not only was it possible to distinguish between samples of the same species, but the two species were separable in this manner (Calaprice MS 1970). The method of X-ray analysis and the equipment used is described in the first report and the statistical techniques are described in the latter, a preliminary report.

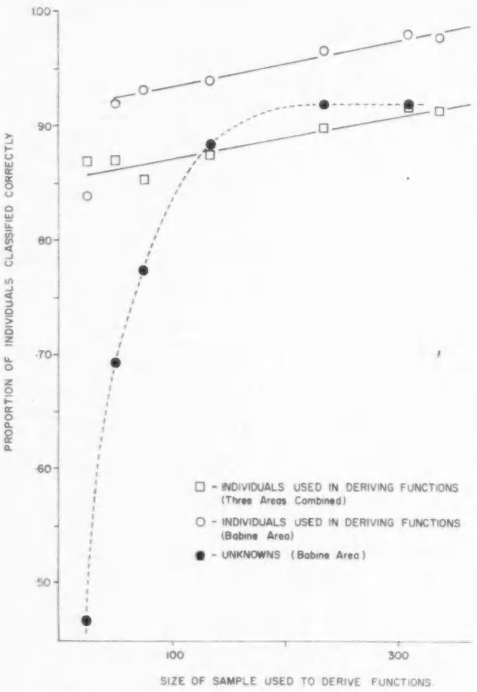

FIG. 2. Classification of individuals using discriminant functions.

In this study, the collections were extended to other life stages of sockeye salmon, the fry and the adult stage. The methods of analysis used were essentially the same with the exception that the samples were exposed to direct radiation from the Americium-241 source instead of secondary radiations from the thulium target mounted in a target assembly described by Giauque (1968). This provided a larger number of X-ray counts for the same counting time, and reduced statistical errors in the chemical analysis. In addition, an automatic sample changer was used that allowed for the processing of 40 samples in an evening. As a result, much of the instability in the electronics observed over long periods of time was reduced and it was not necessary to screen the samples for electronic drift.

The results of this study indicate that samples of fry collected from different areas in the Babine Lake system are recognizably distinct, with few exceptions. The Upper Babine River fish were not distinguishable from the Lower Babine River sample. Separation of samples by use of this method of analysis implies that the chemical composition of the samples is distinct. Failure to detect differences between samples implies that either the chemical composition of the fry is similar, or that the sample sizes are not adequate to demonstrate differences. The similarity between the Upper and Lower Babine River fish may have been predicted, however, for both samples were taken from areas that receive essentially the same water. Both sites are located at the outlet of the Babine Lake system.

The analysis of the tail sections of adult sockeye from different areas gave much the same results as the comparison of fry. There are overall differences among the populations compared and the paired comparisons reveal where the differences exist.

The results of the multiple discriminant analysis of the sockeye fry and adult data demonstrate the potential feasibility of using these techniques to determine the area of origin of individuals taken

by the fishery. In each instance several different discriminant functions were computed and were used to classify individuals forming the samples. In a previous report it was shown that individual salmon smolts could be assigned to their area of origin with no error (Calaprice MS 1970). These analyses were carried out using small samples of fish taken from each locality. This was later found to be somewhat misleading, for it is possible to obtain similar results after the individuals in the different samples were mixed and randomly assigned into groups before the analysis was carried out. Randomizing the individuals among the samples had several recognizable effects upon the results: the F values were not significant, and the proportion of the variance explained by each discriminant function was reduced; however, discrimination was still achieved. I attribute this effect to the large number of variables employed in the analysis and the small sample size.

In this study larger sample sizes were used and the relation between the number of individuals in the sample and the kind of discrimination observed was studied by pooling the Babine Lake groups, and comparing Babine Lake, Cultus Lake, and Adams River fish. The original Babine data was subsampled and the individuals were treated as unknowns. In this manner it was possible to show that an increase in sample size has a marked effect upon the accuracy of classifying unknowns. The accuracy of classifying knowns, i.e., the individuals used to derive the functions, is also increased with increasing sample size and it seems reasonable to conclude that one may achieve as high as 95% correct classification of unknowns providing the sample sizes used to derive the functions are sufficiently large.

The nature of the chemical differences that allow for discrimination is difficult to ascertain. The reasons for this were discussed in detail in an earlier report (Calaprice MS 1970). In summary, differences in the spectra may be due not only to the presence, absence, or concentration of specific chemical elements, but to so-called matrix effects

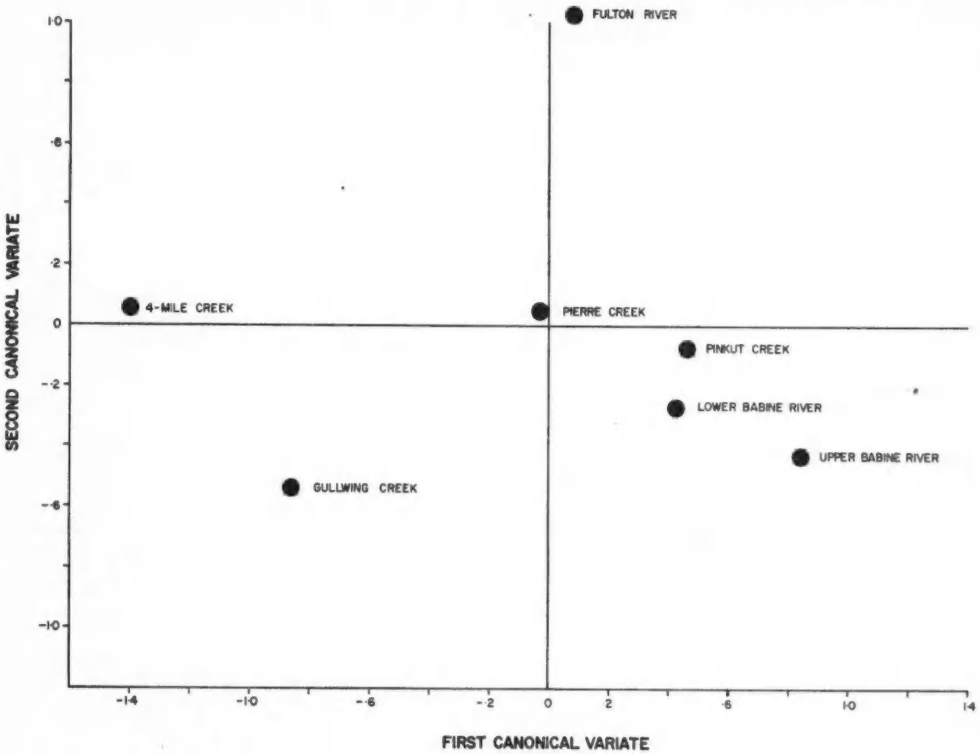

FIG. 3. Canonical analysis of 11 measurements (channels) on sockeye salmon fry.

(Fink et al. 1966; Rhodes 1966). The result is that the chemical differences noted in this and earlier studies may be due to differences in the inorganic, organic, and fine morphological structure of the organism. The results of the stepwise multiple discriminatory analysis demonstrates that a small portion of the spectrum is all that is needed to provide discrimination among certain groups. The first variable is all that is needed to derive a significant function in each instance. However, the addition of more variables renders a better basis for comparison. This is made obvious by counting the number of significant comparisons in each of the various steps in the analysis. The results in Tables 2 and 4 are taken from the last step. There were only 11 significant comparisons after the first stepwise analysis as compared with 19 in the last analysis (Table 2). Furthermore, the significantly different comparisons were made more distinct, for the *P* values were generally smaller in the final step. The X-ray energies associated with each of the variables used in the analysis were obtained by analysing standards. Clark (1963) tabulates the X-ray energies for each element and reference to these tables provides some indication as to the nature of the differences separating groups. Because of the large number of alternatives, additional studies are required to establish with assurance the nature of the chemical differences among groups. These studies are presently underway. However, the same variables that are useful for separating fry are not necessarily the same employed in the separation of adults.

The canonical analysis of the fry and adult data provides a means of comparing the relative similarities among the samples. In this instance the implication is that the closer the points on the graph the more similar the chemistry. The distribution of points in each graph suggested a relation between geographic location and chemical composition. This was also observed in the previous study.

The statistical procedures, the methods, and the underlying assumptions have been discussed elsewhere (Calaprice MS 1970). In that study the results indicated that not only might populations of smolts be usefully separated but the two species were separable as well. It was not known at that time whether such discrimination could be achieved with adults. The results of this study demonstrate

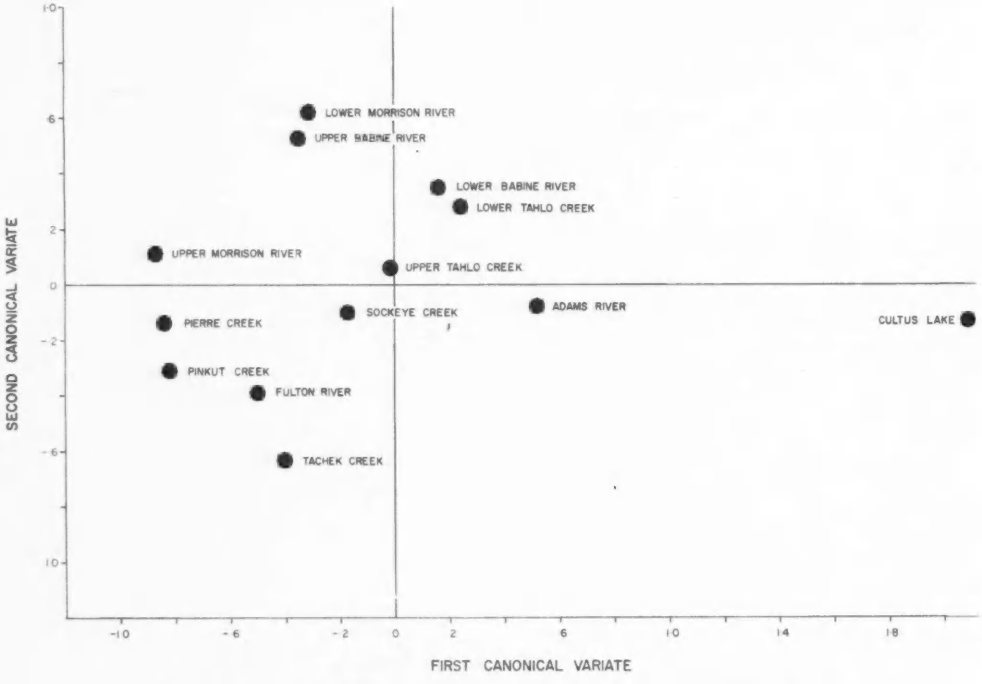

FIG. 4. Canonical analysis of nine measurements on sockeye salmon adults.

TABLE 5. Summary — stepwise discriminant function analysis.

Step no.	Variable no.	No. variables included in function	Probability for variable	U statistic	Approximate F	Associated probability	Energy (keV)
			Fry				
1	22	1	<.0001	.8123	5.50	.00005	28.4
2	20	2	.0207	.7322	3.99	.00001	26.8
3	9	3	.0160	.6565	3.56	<.00000	18.2
4	26	4	.0418	.5988	3.23	<.00000	31.5
5	1	5	.0464	.5469	3.03	<.00000	11.9
6	2	6	.0002	.4519	3.35	<.00000	12.7
7	4	7	.0959	.4183	3.14	<.00000	14.3
8	8	8	.0324	.3786	3.06	<.00000	17.4
9	12	9	.0242	.3576	3.26	<.00000	20.5
10	13	10	.0565	.3270	3.14	<.00000	21.3
11	10	11	.0441	.2974	3.07	<.00000	18.9
			Adults				
1	17	1	<.0000	.8602	5.15	<.00000	24.4
2	4	2	<.0000	.6610	7.26	<.00000	14.3
3	13	3	.0334	.6235	5.38	<.00000	21.3
4	1	4	.0230	.5861	4.51	<.00000	11.9
5	6	5	.0000	.4866	4.89	<.00000	15.8
6	16	6	.0144	.4555	4.42	<.00000	23.7
7	22	7	.0325	.4293	4.05	<.00000	28.3
8	38	8	.0258	.4037	3.79	<.00000	40.9
9	33	9	.0888	.3839	3.54	<.00000	37.0

that certain populations of adult sockeye are distinguishable. Thus it seems likely that these techniques may be useful in determining the geographic origin of sockeye caught in the fishery.

Acknowledgments

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Dissolved Oxygen Requirements for Embryonic and Larval Development of the Hardshell Clam, *Mercenaria mercenaria*

GEORGE MORRISON

National Marine Water Quality Laboratory
Water Quality Office, Environmental Protection Agency, West Kingston, Rhode Island 02892

MORRISON, G. 1971. Dissolved oxygen requirements for embryonic and larval development of the hardshell clam, *Mercenaria mercenaria*. J. Fish. Res. Bd. Canada 28: 379–381.

Eggs and larvae of *Mercenaria mercenaria* were exposed to various levels of dissolved oxygen for varying time periods. Larval growth was normal in levels of dissolved oxygen at or above 4.2 mg/liter, but was essentially curtailed in levels at or below 2.4 mg/liter. Larvae survived extended exposures of 1 mg/liter, but showed little growth. Growth rates of larvae transferred to conditions of high dissolved oxygen after various periods of exposure to low dissolved oxygen became normal. Eggs developed normally at oxygen levels down to 0.5 mg/liter. One hundred percent mortality was experienced at 0.2 mg/liter.

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THE hardshell clam, *Mercenaria mercenaria*, is typically an estuarine organism and is, therefore, subject to the wide-ranging environmental conditions of that habitat. Although the effects of temperature, salinity, turbidity (Loosanoff and Davis 1963), and *p*H (Calabrese and Davis 1966) have been well documented, there have been no investigations of the oxygen requirements of the eggs and larvae[1] of *M. mercenaria*. The present study was, therefore, designed to determine the following: (1) the minimum level of dissolved oxygen required for normal egg development; (2) the effect of continually reduced oxygen levels on larval growth; and (3) the effect of short-term low oxygen stress on the subsequent growth of larvae.

Methods and Materials

The experimental apparatus (Fig. 1) provided a series of stable reservoirs of dissolved oxygen at levels ranging from 0.2 to 6.5 mg/liter. Filtered sea water at ambient salinity (28–30‰) was heated to 25 ± 1 C and passed through a 1.3-cm diam PVC column. The water was purged of dissolved oxygen by introducing nitrogen through an air stone at the bottom of the column. The flow of nitrogen, although sufficient to lower the oxygen level, did not alter the *p*H or have any apparent effect on egg and larval development as evidenced by the experimental controls. The deoxygenated water was sequentially reaerated by flowing it at approximately 250 ml/min over a series of baffles open to the atmosphere.[2]

Water was maintained at desired oxygen levels in each of four test chambers by siphoning it from selected points along the trough and introducing it at the bottom of the test chambers, at a rate of approximately 20 ml/min. The test chambers used for rearing eggs and larvae were 2-liter pyrex aspirator bottles. An inverted funnel, covered with a 53-μ nylon screen to prevent the loss of larvae, provided the overflow from each chamber.

EMBRYOLOGICAL DEVELOPMENT

The methods used for obtaining viable gametes were those described by Loosanoff and Davis (1963). Since preliminary experiments suggested that embryological

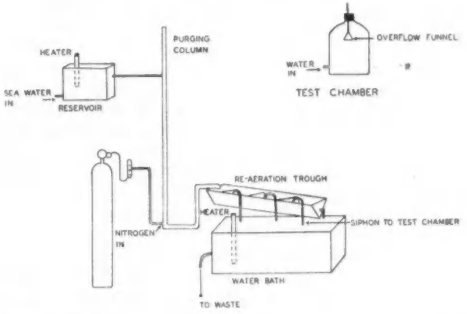

FIG. 1. Diagram of dissolved oxygen apparatus.

[1]Larvae, as used in the paper, refers to the shelled veliger stage unless otherwise designated.

[2]This dissolved oxygen apparatus evolved from one of similar design in use at the National Water Quality Laboratory, Water Quality Office, Environmental Protection Agency, Duluth, Minnesota.

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development was not affected at the 1 mg/liter dissolved oxygen level, the tests were conducted at 0.2, 0.5, and 1.0 mg/liter of dissolved oxygen respectively, and a high oxygen control level of 5.5 mg/liter was used to verify initial egg quality. When the number of eggs developing into abnormal larvae, using the criteria devised by Woelke (1961), exceeded 10% in the 5.5 mg/liter level, the entire test was discarded. Fertilized eggs (15 eggs/ml) were held in the chambers containing static (nonflowing) sea water drawn from the test apparatus at the desired oxygen concentration. These chambers were incubated at 25 C, midpoint in the range of 17.5–32.5 C for normal egg and larval development (Matthiessen and Toner 1966). The ratio of test water volume to egg concentration was sufficient to maintain the dissolved oxygen level within 0.1 mg/liter of the desired concentration during the 24-hr test period. The percentage of eggs that successfully developed to the straight-hinge veliger stage was determined for each oxygen level approximately 24 hr after fertilization. Each of the four oxygen levels for a given experiment were tested simultaneously, with two replicates and with eggs from a specific adult.

Larval Development

Four experiments of 14 days duration each were conducted to evaluate the effects of various dissolved oxygen concentrations on larval growth. Since preliminary studies indicated that larvae survived but did not develop normally at the 1 mg/liter dissolved oxygen level, four oxygen concentrations ranging from 1.0 to 5.5 mg/liter at 1.5-mg/liter intervals were selected as the test concentrations. The mean dissolved oxygen concentrations for all the tests, however, were 0.9 ± 0.3, 2.4 ± 0.4, 4.2 ± 0.4, and 5.6 ± 0.5 mg/liter. Fertilized eggs (15 eggs/ml) were added to test chambers adjusted to the desired oxygen concentration. Water flow through the chambers (20 ml/min) was begun after the larvae had reached the straight-hinge stage (approximately 24 hr). The number of eggs developing into normal straight-hinge larvae was the same in each test chamber, as the dissolved oxygen levels (1–5.5 mg/liter) were above the minimum required for normal embryological development. The larvae at all oxygen concentrations were fed equal amounts of laboratory-grown phytoplankton cultures (*Isochrysis galbana* and *Monochrysis lutheri*) three times a week. The addition of the photosynthetic food supply caused slight fluctuations in the oxygen concentrations but these were generally less than 0.5 mg/liter and always returned to the levels before phytoplankton addition within 3 hr. Dissolved oxygen concentrations were measured daily by filling a small vial from the overflow tube and using a Delta oxygen meter, Model 85, calibrated by the standard Winkler oxygen determination. Larval growth was measured twice a week by measuring the shell length of 50 individuals on an axis parallel to the hinge.

Latent effects of exposure to low levels of dissolved oxygen on subsequent larval development were determined by holding newly developed larvae for 6 days in static sea water adjusted to 1 mg/liter of dissolved oxygen. Approximately 300 larvae were removed every 24 hr and placed in oxygen-saturated water (~6.0 mg/liter). Dissolved oxygen and growth measurements were taken daily. Larvae were fed *I. galbana* and *M. lutheri* after being placed in high oxygen water.

Results

Embryological Development

Embryological development was normal from 0.5 mg/liter and above. One hundred percent mortality occurred at the 0.2 mg/liter oxygen level. It appears that the majority of the eggs reached at least the 64-cell stage before mortality. In a single separate experiment, eggs held at 0.34 mg/liter developed to the trochophore stage but did not form shells.

Larval Development

Growth rates of *M. mercenaria* larvae vary directly with the dissolved oxygen concentrations used in this test (Fig. 2). A distinct break falls between the 2.4 and 4.2 mg/liter dissolved oxygen levels. Growth is approximately 0.9 microns/day for the larvae in both the 0.9 and 2.4 mg/liter oxygen levels; 4.0 microns/day in the 4.2 mg/liter oxygen concentration; and 5.0 microns/day for those in the 5.6 mg/liter dissolved oxygen water.

There appears to be no permanent effect due to short-term low oxygen stress during early developmental stages on subsequent larval growth. Figure 3 shows that growth rates become normal when larvae are placed in high-oxygen water.

The larval growth rates were lower for those organisms held in the flow-through chambers (Fig. 2) than those in static conditions (Fig. 3), the probable reason being that the food concentration available to the larvae would be less in a flowing system where the small phytoplankton

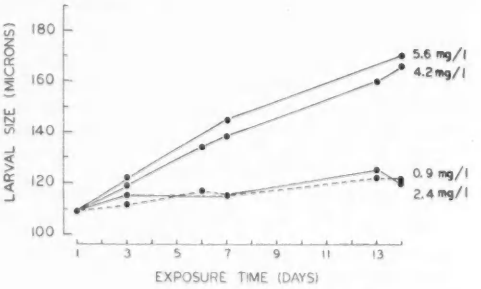

FIG. 2. Growth rates of larvae held in flowing sea water at four dissolved oxygen levels.

cells were constantly being lost through the overflow screen.

Discussion

Larval development of *M. mercenaria* is markedly affected through stress induced by low levels of dissolved oxygen. If larvae were to continue to grow at the same rates indicated in Fig. 2, those from the 0.9 and 2.4 mg/liter dissolved oxygen levels would take approximately 60 days to reach setting size as compared with 15–20 days for those from the 4.2 to 5.6 mg/liter oxygen levels. The expected duration of the planktonic stage is 20 days in nature (Tubiash et al. 1965). Prolonged exposure of larvae to oxygen levels below 4 mg/liter would be detrimental to the extent that it would lengthen the planktonic stage of the organism's life cycle and thereby decrease its probability of survival.

Development of *M. mercenaria* eggs to the straight-hinge stage is affected only at low dissolved oxygen concentrations. Whereas larval growth was essentially halted at levels between 2.4 and 4.2 mg/liter, egg development was not arrested until oxygen levels fell between 0.2 and 0.5 mg/liter, roughly one order of magnitude in difference. It is thus evident that low dissolved oxygen levels, though adequate for egg development, would prohibit production and growth of the larval stages of *M. mercenaria*. This illustrates the need to know environmental requirements for all stages of development in the life cycles of marine organisms.

Further studies are needed to determine what effects, if any, exposure to low dissolved oxygen (4 mg/liter or less) conditions would have on subsequent developmental stages of *M. mercenaria* in respect to growth, sexual maturation, spawning, and successful development of the F_2 generation.

Short-term stress by low levels of dissolved oxygen has no apparent lasting effect on *M. mercenaria* larval development. Growth rates become normal and metamorphosis occurs when larvae are placed in high oxygen water.

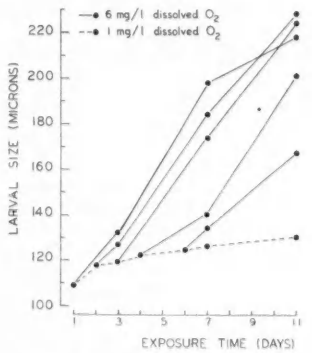

FIG. 3. Growth rates of larvae in high oxygen water after transfer from low oxygen conditions. The broken line represents growth at 1 mg/liter and the solid lines growth at 6 mg/liter dissolved oxygen.

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I am greatly indebted to Dr R. Steele for the supply of algal cultures, to Miss D. Black and Mr W. Giles for their technical assistance, and to Dr D. K. Phelps for his support and encouragement during this study.

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High Tide Movements and Homing Behaviour of the Tidepool Sculpin *Oligocottus maculosus*[1,2]

JOHN M. GREEN

Department of Biology and Marine Sciences Research Laboratory
Memorial University of Newfoundland, St. John's, Nfld.

GREEN, J. M. 1971. High tide movements and homing behaviour of the tidepool sculpin *Oligocottus maculosus*. J. Fish. Res. Bd. Canada 28: 383–389.

Tagging studies show that the tidepool sculpin *Oligocottus maculosus* Girard shows fidelity to specific tide pools and will return to their pools when displaced. The results indicate that the navigational ability of *O. maculosus* is not solely dependent upon familiarity with geographical features of the intertidal zone. It is suggested that homing behavior functions as a mechanism stabilizing the spatial distribution of this species.

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THE movements at high tide of *Oligocottus maculosus* Girard at different shore sites with various exposures to surf, and during different seasons of the year were investigated, and the relation of individual fish to particular tide pools was determined through tagging. Observations and experiments were conducted to determine the factors that affect the tendency of *O. maculosus* to return to specific pools. The study was conducted on the southwest coast of Vancouver Island, B.C., at a site approximately 5 km southwest of Port Renfrew and 400 m southeast of the entrance to San Juan Inlet.

Methods

The high tide distribution of *O. maculosus* was determined by direct observations of tagged (see below) and untagged specimens. Most observations had to be made from beneath the water surface except at times of calm seas. Most underwater observations were made by skin diving but sometimes scuba was used. During stormy weather, particularly during the winter months, it was necessary to confine the observations to the relatively sheltered sites. In one moderately sheltered area monthly observations were made to determine the number of *O. maculosus* at high tide in an area of approximately 8 m² surrounding a single tide pool. Collections were also made with wire minnow traps on a monthly basis and it was therefore possible to calculate the number of fish in the area surrounding the tide pool as a percent of total population of the pool.

Monofilament gillnets of 13 mm mesh placed at various depths and sites in the intertidal zone were also used to assess distributions at high tide. Each net was mounted on two steel rods 61 cm long, 13 mm diameter (Fig. 1). The rods were threaded at the bottom end and screwed into 13-mm Philip shields anchored in the substratum. Three shields were placed 2 m equidistant from one another at each gillnet site. Thus, all three sides of a triangle could be closed off with netting. The net was hung between the rods and weighted so its bottom lay along the contour of the shore. Nets were examined for fish either just before or just after the ebbing tide exposed them.

TAGGING

Tags, consisting of one or two colored embroidery beads, were attached with monofilament nylon line to the fish's dorsal musculature midway between the anterior and posterior dorsal fins. Finclipping was done in conjunction with bead tagging so that individual fish could be identified. One or both pelvic fins, or in some instances anterior or posterior portions of the anal or dorsal fins, were clipped. Fish were caught for tagging with baited minnow traps or with small dipnets. No anesthetic was used and each fish was sexed and measured after tagging.

Two tests were used to evaluate the effects of tagging on survival. Over a period of 18 months 25 bead-tagged *O. maculosus* were held in aquaria with 20 nontagged *O. maculosus*, 55–70 mm long, each for a minimum period of 6 months. The water in the aquaria was not circulated or filtered, but was renewed every 7 or 10 days. Daily temperature fluctuations in the aquaria corresponded to those that occur in tide pools of the upper intertidal zone. There were no mortalities of either control or tagged fish.

In order to more accurately simulate natural conditions, 10 bead-tagged *O. maculosus* (60–70 mm) and 8 nontagged *O. maculosus* (63–71 mm) were held in an 80-liter covered tide pool at the 2.7-m tide level from November 10, 1965, to June 9, 1966. One died

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FIG. 1. Gillnet site showing the three equidistant supporting rods anchored in the substratum.

as a result of being caught under the screen cover and another lost its tag between March 25 and 30, but was in healthy condition with the wound completely closed when the experiment was terminated. The remaining tagged fish were in good physical condition at the end of the experiment. The flesh surrounding the tags in these fish was worn away to varying degrees and the tags of several fish were about to be lost.

The latter observations indicated that tag loss could occur in the pool environment in a matter of months or even weeks. Since it was no more likely for tags to become caught on objects in the covered pool than in the aquaria, tag loss was probably due to the abrasive action of turbulent water, a conclusion supported by the observation that fewer tags were lost during the summer than winter.

Tag loss apparently does not result in increased mortality. Bead tags were forcefully removed from 10 *O. maculosus* 1 week after they had been attached. Five of these fish were held in an aquarium and five were held in the covered pool. At the end of 4 weeks no deaths had occurred and the wounds of all the fish were healing.

TYPES OF TAGGING EXPERIMENTS

Two types of tagging experiments were conducted: transplant releases and home pool releases. In the former, fish from one tide pool were tagged and subsequently released elsewhere — usually into another pool, but sometimes directly into the sea. In the latter, tagged fish were released into the same pool in which they were captured.

Most recovery data were obtained by trapping of pools with minnow traps and from nocturnal observations of pools. The latter method proved to be extremely effective, because tidepool sculpins leave their hiding places at night and position themselves in the open, and can be easily seen and captured with a small hand net. Consequently, a large number of pools can be examined in a relatively short time.

Results

HIGH TIDE DISTRIBUTION AND MOVEMENTS

On the basis of diving observations and gillnet catches, it was found that the high tide distribution

of *O. maculosus* depends upon both space and time factors. Along the shore there is a difference between the high tide distribution of fish inhabiting exposed and those inhabiting sheltered tide pools. In the former habitat *O. maculosus* does not regularly leave the tide pool whereas in the latter it does. The seasonal change in the high tide distribution of fish inhabiting tide pools of intermediate exposure is shown in Fig. 2. The only time a large proportion of the population was out of the pool was during July, August, and September.

In transects where the high tide distribution encompasses a larger area than the low tide distribution, the species does not exhibit a complete upward shift in its vertical distribution. Some fish always remain in the tide pool or at the same tide height as the pool. The only specimens that show a more or less complete tidal shift in their vertical distribution are 0-year fish in some sheltered and moderately sheltered areas. If there are no physical impediments these fish migrate shoreward as their pools are flooded and then move offshore again as the tide ebbs. This onshore, offshore migration has often been observed in an area where a large sheltered tide pool at the 2.1-m tide level has only sand and gravel shoreward of it.

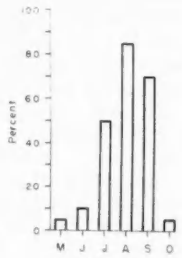

FIG. 2. Percent of the total tide-pool population of *O. maculosus* out of the tide pool at high tide during the months that this occurred. The value for each month represents the average for at least six high tides.

According to the above observations, during certain months of the year the same individuals will be in particular tide pools on successive days or weeks. Data from 117 tagged *O. maculosus* released into the tide pools from which they were taken supports this (Table 1).

In addition to obtaining data by trapping tide pools, together with night observations at low tide, high tide observations were made on pools in which tagged fish were released. When sea conditions permitted, broken pieces of fresh *Mytilus californianus* were placed at the bottom of tide pools at or near the time of high tide. This attracted the sculpins in the pool and also brought the tagged specimens into view. The number of tags observed could then be compared with the number of tagged fish released into the pool. This confirmed that in exposed pools throughout the year, and in tide pools of intermediate exposure during most of the year, nearly all *O. maculosus* remain in the pools while they are flooded.

The movements of fish to and from tide pools with intermediate exposure showed marked individual differences. Several specimens watched three times a week for a month were never seen beyond 1–2 m from the edge of their pool, in an

TABLE 1. Summary of nine experiments on home pool releases of *O. maculosus*. Recoveries within the first 2 weeks of tagging are not included.

	No.							
	tagged		recovered		recovered in home pool		% resident of	
Date	♀	♂	♀	♂	♀	♂	recovered	tagged
22-X-65	9	13	6	12	6	12	100	82
23-X-65	4	3	4	2	4	2	100	86
22-VI-66	3	1	3	1	2	1	75	75
18-VII-66	22	22	21	21	21	21	100	96
8-IX-66	3	4	3	4	3	4	100	100
8-IX-66	2	2	1	2	0	2	50	50
9-X-66	3	2	3	2	2	2	80	80
10-X-66	0	5	0	5	0	5	100	100
25-XI-66	12	7	10	3	10	3	100	68
Totals	58	59	51	52	48	52	94 100	83 88
	117		103		100		97	86

area of over 250 m^2 located on a flat shelf. Tagged fish from other pools on the same shelf 3–5 m away would frequently move into the vicinity of this particular tide pool, but none moved more than 10 m from its home pool. On this shelf, movement or vision was not restricted.

Duration of Residence in Specific Tide Pools and Straying

The length of time that individuals spend in one tide pool cannot be calculated from decreases in tag returns because tag loss is a significant factor. It is also possible that tagged fish may be more susceptible to predation, at least in certain pool areas. Duration of residence in specific tide pools was determined from data on fish which were identifiable for long periods as a result of clipped fins or other morphological characteristics. Thus 40 *O. maculosus* were watched in the same pool for over 6 months, and 10 for more than a year. From this it may be concluded that the majority of *O. maculosus* over 55 mm in length permanently inhabit specific pools in exposed and moderately exposed areas.

Some marked fish have been observed in two or more pools. For example three fish that were tagged and returned to the home pool were retrapped in other pools, but never again in the home pool. Also, 10 fish that were in the home pool at least 2 weeks after being tagged were later seen elsewhere.

Homing Behaviour

Williams (1957), in his study of homing behaviour of rocky shore fishes, described the presence of the same fish in the same pool at two different low tides as an instance of homing. Homing should indicate that a species has navigational abilities rather than just fidelity to a particular geographical site. Consequently, on the basis of what has been stated about the high tide distribution of *O. maculosus*, homing must be described in terms of the return of individual fish to tide pools from which they have been displaced. Homing experiments, therefore, consisted of tagging fish from specific pools and releasing them at sites other than the home pool. Only the first recapture of the fish in the home pool was considered to be an instance of homing.

Homing success

The transplant experiments were carried out in the same general area as the home pool release experiments and in many instances in the same pools. From the 21 experiments, using 149 *O. maculosus* (Table 2), it is apparent that this species possesses strong homing behaviour, and that they can return to the home pool after being displaced over relatively long distances (Fig. 3).

Males and females showed no differences in the homing behavior and age differences were unlikely (Table 3).

Behavior of transplants

Often unfavorable conditions prevented a search of the home pool at the low tides immediately after the release of transplanted fish. Therefore, the time span required for successful homing could not always be established. Complete data on nine fish suggest that most successful homing is accomplished within the first one or two high tides after release.

In three instances a thorough search was made of a pool during the first low tide after the release of transplanted fish into it. This showed that the fish did not remain in the pool into which they were transplanted. General observations and trap catches indicated that only four of over 100 transplants were still in the transplant pool after the first high tide, and two of these remained there for 2 weeks and then returned to their respective home pools. A third fish remained in the transplant pool for 4 weeks before returning to its home pool; the fourth was poisoned in the transplant pool on the first low tide after its release.

The prompt departure from the transplant pool was not related to the density of *O. maculosus* or other sculpins in the transplant pool. Removal by poisoning or trapping of a part or all of the fish in a pool several days before transplanting fish into it did not result in the transplanted fish remaining there a longer time. Reciprocal transplants in which the same number of tagged fish were interchanged between two pools also did not modify this behaviour.

Possible Influencing Factors

Position of pools

In the transplant experiments fish were moved to various distances from the home pools. In some experiments the home and the transplant pools were at about the same tide level, whereas in others they ranged over the entire vertical distribution of *O. maculosus*. At times the two pools were both vertically and horizontally separated from each other. Comparisons of individual experiments indicate that the vertical and horizontal positions of the pools do not influence homing success.

TABLE 2. Summary of 21 experiments on transplant releases of *O. maculosus*.

Date	No. tagged ♀	No. tagged ♂	No. recovered ♀	No. recovered ♂	No. recovered in home pool ♀	No. recovered in home pool ♂	% homed of recovered	% homed of tagged
22-X-65	6	6	5	6	5	5	91	83
22-I-66	9	2	6	1	5	1	86	55
20-II-66	3	3	3	3	3	3	100	100
2-III-66	2	3	1	2	1	2	100	60
30-IV-66	0	7	0	7	0	7	100	100
2-V-66	7	9	5	6	5	6	100	69
10-VI-66	4	2	4	2	3	2	83	83
16-VI-66	3	4	3	4	2	3	71	71
16-VI-66	3	6	1	6	1	4	71	56
22-VI-66	0	2	0	1	0	1	100	50
24-VIII-66	5	4	5	3	5	3	100	89
2-IX-66	2	1	2	1	1	1	67	67
2-IX-66	3	0	1	0	1	0	100	33
25-IX-66	7	1	5	1	3	0	50	38
27-IX-66	3	1	2	0	2	0	100	50
27-IX-66	2	3	2	0	1	0	50	20
1-XI-66	2	2	2	2	2	2	100	100
9-XI-66	3	2	3	1	3	0	75	60
9-XI-66	0	5	0	4	0	4	100	80
9-XI-66	2	3	2	0	2	0	100	40
9-XI-66	5	5	4	3	4	3	100	70
Totals	71	71	56	53	49	47	88 89	69 66
	142		109		96		88	68

Distance from home pool

Some of the fish in transplant experiments summarized in Table 2 were moved as much as 102 m from their home pool. Although the greatest transplant distance from the home pool was within the homing ability of the species, homing success does appear to be related to the transplant distance (Fig. 3). This may not only reflect the effects of distance but also of terrain topography. In the experiment where high degrees of homing success were observed over a distance of 102 m, the fish moved across a relatively level portion of the shore.

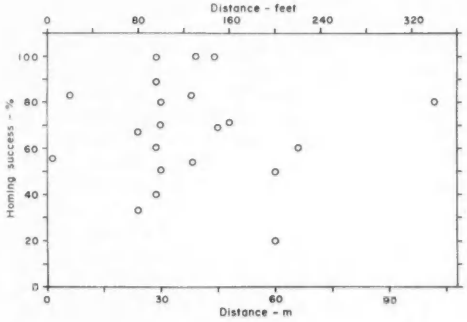

FIG. 3. Homing success of transplanted *O. maculosus* as a function of the distance away from the home pool where they were released.

TABLE 3. Summary of 20 transplant experiments of *O. maculosus* showing the homing success of different size groups.

Size groups (*mm*)	No. tagged	No. recovered	No. recovered in home pool	% homed of tagged
50–54	5	5	4	80
55–59	9	6	6	67
60–64	22	16	15	68
65–69	28	25	22	79
70–74	27	22	19	70
75–79	17	13	11	65
80–84	19	16	16	84
85–90	5	4	3	60

Three transplant experiments that are not included in Table 2 indicate that *O. maculosus* will move over considerable distances in search of the home pool. In two experiments, 20, and in a third 10, specimens were tagged and moved to a pool approximately 240 m away. Extensive trapping and night observations of pools within 60–90 m of the transplant pool indicated that all the displaced fish moved out of the study area. Unfortunately, it could not be determined if any of these fish successfully homed.

Time of year

Homing success as a function of the season is shown in Fig. 4. It is important to note that *O. maculosus* exhibits homing behaviour throughout the year. The fact that all these experiments were conducted on a moderately exposed to exposed shore means that homing occurs during the season when the fish are seldom, if ever, out of the home pool.

Captivity

Nearly all transplanted fish were held in aquaria for the duration of at least one tide cycle before being released. In five experiments (25 specimens) fish were held for three tide cycles. Homing success in these five experiments was not lower than the average success of all the other experiments.

In one instance a tagged fish held in a covered pool escaped after being in the pool for 6 months. This fish was subsequently trapped in its home pool. It remained in this pool for at least 2 months before disappearing.

The movement of fish out of an unfamiliar pool on the first tide that floods the pool appears to occur regardless of how long the fish have been held in captivity. In one of the two experiments described above in which 20 *O. maculosus* ,were transplanted from a pool 240 m from the study area, the fish were held for 2 weeks before being released. They left the transplant pool on the first tide that flooded it. Similar results were obtained in another experiment involving six tagged fish from a single pool that had been held for 6 months in an aquarium with six tagged fish from another part of the study area. At the end of 6 months all 12 fish were released into the home pool of the first six. Extensive trapping a week later showed that the six fish from that pool were still there, but that none of the other six fish were.

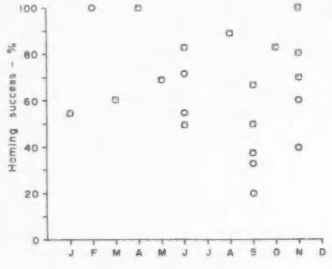

FIG. 4. Homing success of transplanted *O. maculosus* as a function of the month during which the transplants were made.

Discussion

The consequence of restricted movements of a tidepool sculpin as the result of specific exposure characteristics of the home pool raises doubts about the validity of attributing homing behaviour to a species only on the basis of the presence of the same fish in a pool on consecutive low tides. Although concluding that *Clinocottus analis* homes on the basis of marked fish released into their home pool, Williams (1957) showed that this species will not return to the home pool when displaced to pools 2 m or more away. He attributed this to the fact that disturbance of the fish during capture and marking causes a considerable portion to abandon their home pool, and that their bottom-dwelling habits provide a limited view of the surrounding areas. How this latter statement is reconciled with the conclusion that the species homes as a result of familiarity with geographical cues outside the pool is not clear. Disturbance during capture and tagging of *O. maculosus* does not appear to affect homing behaviour or the fidelity of an individual to the home pool. *Oligocottus maculosus* is also a bottom-dwelling species but will return to the home pool over considerable distances.

Displacement experiments with *Blennius pholis* and *Enophrys bubalis* did not give evidence for a directed movement back to the original pool (Gibson 1967). Conversely, *O. maculosus* does appear to exhibit directed movements back to the home pool. The mechanism by which these movements are made is not known, but certainly homing is dependent upon a precise behavioural mechanism. It appears also that this mechanism does not necessitate a spatial familiarity with the area covered during homing. In some areas *O. maculosus* exhibits regular movements away from the home pool, but even these movements are restricted when compared with the distance over which the species will home. If individuals can recognize geographical features beyond the range of their daily movements they must have obtained this information at an

age and size when they do not show strong fidelity to specific pools.

Oligocottus maculosus has been shown to home to pools after being held in an unnatural environment for 6 months. This indicates that an "image" of the home pool is retained over long periods, and suggests that at some stage in the development of individual fish some pool characteristic(s) is(are) learned. Unless information relating to the geographical features of the surrounding area is similarly learned, it is difficult to avoid the conclusion that this species has bicoordinate navigational ability. Whether *O. maculosus* does orientate more or less directly toward the home pool is not known. The transplant experiments indicate that it at least orientates in the right direction relative to the position of the shore. Once an individual is swimming in the proper direction parallel to the shore, orientation to a specific depth of water or familiarity with a transect perpendicular to the shore could then provide the cues that would enable the individual to home.

According to Williams (1957) behaviour in intertidal fish is "a mechanism by which shallow water fishes of rocky shore areas avoid being left by the tide in unfavorable situations at low tide, such as pools that disappear through sub-surface drainage." If there is danger of *O. maculosus* being left in unfavorable situations at low tide, the homing instinct would be a mechanism of survival to the species. One is left with the question of whether or not such dangers were the primary cause for the development of homing behaviour and whether this is the primary function it now serves. It would appear, for example, that familiarization with the biotic features characteristic of favorable tide pools would prevent the fish from being stranded in a nontide-pool location, and would not necessitate the return to a home pool. Under conditions of high turbulence, the apparently inflexible homing behaviour in *O. maculosus* would seem to be a liability rather than an asset.

Homing behaviour in *O. maculosus* would appear to serve much the same purpose that territoriality serves in other species. Gerking (1959) states that "territoriality is a stabilizing influence because it separates individuals from one another in a regular and orderly fashion in addition to making the fish intimately aware of its surroundings." Territoriality depends upon aggressive interaction among individuals in a population. As there is no aggressive interaction among individuals of *O. maculosus* (Green 1971) territoriality in the above sense does not exist in this species.

It would seem, however, that in a species like *O. maculosus*, which inhabits an extremely variable and rigorous environment, a strong mechanism by which the species remains dispersed and stabilized would be of special importance and adaptiveness. In the absence of such a mechanism the species would tend to aggregate in only the most favorable habitats — the tide pools — within its range of distribution. Aggregated in this way the species as a whole would be more susceptible to the unpredictability of environmental conditions typical of an exposed coast. The learning of visual cues of the tide pool by an individual at an age when the juvenile population is relatively well distributed throughout the range of the species, coupled with homing behaviour is visualised as serving this stabilizing function in *O. maculosus*.

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The Red-Water Ciliate *Mesodinium rubrum* and its "Incomplete Symbionts": A Review Including New Ultrastructural Observations

F. J. R. TAYLOR AND D. J. BLACKBOURN

Institute of Oceanography

AND JANICE BLACKBOURN

Department of Anatomy
University of British Columbia, Vancouver 8, B.C.

TAYLOR, F. J. R., D. J. BLACKBOURN, AND J. BLACKBOURN. 1971. The red-water ciliate *Mesodinium rubrum* and its "incomplete symbionts": a review including new ultrastructural observations. J. Fish. Res. Bd. Canada 28: 391–407.

The authors provide the first ultrastructural description of the marine holotrich ciliate *Mesodinium rubrum* and of its unusual "incomplete symbionts," consisting of numerous functional chloroplasts associated with distinctive nonciliate mitochondria but apparently lacking associated nuclei. The pigment characteristics suggest a cryptomonad origin for the chloroplasts but they are traversed by lamellae that are either ungrouped or in groups of three's, this being contrary to ultrastructural observations on cryptomonads to date. Unusual ultrastructural features of the ciliate include the lack of alveoli in the very thin pellicle and two types of ciliary differentiation: the presence of lateral packing material in some of the cilia grouped to form cirri, and narrow distal portions from which the nine peripheral microtubule duplets are excluded. The cytostome is greatly reduced and the ciliate usually swims backwards. The problem as to the degree of permanence reflected by these features, including whether the symbiotic state is obligate or not, was not resolved. The authors discuss the evidence bearing on this question.

The paper also reviews the ecology and taxonomy of the ciliate. Fifty instances of its occurrence, often in conjunction with nontoxic "red water," are summarised. The species has an extremely wide geographic distribution, including both polar and equatorial waters. Red water caused by the species occurs either within extreme coastal localities (bays, inlets) or in the vicinity of offshore upwelling. Field studies demonstrated that the ciliate–chloroplast consortium is a functional autotroph capable of playing the role of primary producer.

Taxonomic difficulties are summarised. Previous designations of the species to the genus *Cyclotrichium* are considered inappropriate. Detailed study of widely spread populations is needed to determine the degree of taxonomic confusion involved.

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IN Charles Darwin's account of the voyage of the *Beagle* (1839) there is a detailed description of microorganisms that caused an extensive reddish discoloration of the sea off southern Chile. Despite the lack of an illustration his description is so graphic that Hart (1943) was convinced that the species involved was *Mesodinium rubrum* (Lohmann), an opinion with which we concur.

In fact, although *M. rubrum* has apparently been observed on many occasions[1] and often in bloom conditions, relatively little has been added to the description of Darwin, with the notable exception of Bary and Stuckey's (1950) preliminary cytological study. Further, taxonomic confusion surrounds the species. As the species has not been cultured, definitive studies on its unusual symbiotic condition have not been possible.

Recently we reported on the preliminary results of an electron microscope examination of the

[1]As a matter of historical curiosity *M. rubrum* could conceivably qualify as the first marine protist observed by Anthony van Leeuwenhoek: "July 27. 1676. I went to the Sea-side, at Schevelingen... and viewing some of the Sea-water very attentively... I saw in it a little animal that was blackish, looking as if it had been made up of two globules. This creature had a peculiar motion, after the manner as when we see a very little flea leaping upon a white paper [yet at every leap it moved only about the length of a coarse sand-grain]; so that it might very well be called a Water-flea; but it was by far not so great as the eye of that little animal, which Dr. Swammerdam calls the Water-flea." [*Daphnia*] (Translation from Committee of Dutch Scientists 1941, letter no. 26; see also Dobell 1932, letter no. 18.)

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chloroplasts and associated structures within the ciliate (Taylor et al. 1969). We now summarise the existing information on this ciliate, clarifying some points of taxonomic confusion and including the full results of our ultrastructural observations.

The descriptive sections that follow assume synonymy between *M. rubrum* and *Cyclotrichium meunieri* Powers. *Mesodinium pulex* (Claparède et Lachmann) is considered a separate species but has apparently been confused with *M. rubrum* in a number of instances. The reasons for these opinions are given in the taxonomic section that follows later.

The Living Organism

Cells of *M. rubrum* behave in a characteristic fashion much as Van Leeuwenhoek and Darwin described. Our high power examinations of living material were performed without a coverslip as even slight pressure can cause the cells to burst.

One of the most striking features of the living cells is an equatorial ciliary belt (ECB). Cilia in this belt arise in a broad band around the cell, most commonly equatorial in position, but due to differences in cell shape (Fig. 3–5) it may lie more anteriorly. The cilia of the ECB are directed forwards in a thick corona surrounding the anterior of the cell. Depending on the degree of development of the anterior portion of the cells the corona may extend beyond the anterior pole or simply surround the sides. It is strikingly refractile in appearance, imparting a "halo-like" appearance to the anterior end and was misinterpreted by some early authors as a lorica type of structure. In optical section it can be observed to be quite thick near the ciliary origin, tapering distally. Careful surface examination reveals that the cilia of the ECB curve towards the right (the organism's left) as they proceed towards the apex. Bary and Stuckey (1950) figured a specimen in which these cilia are wound to the animal's right (their fig. 1). All the specimens examined by us show laeval torsion, a feature also found by Fenchel (1968a, b) who remarked on this discrepancy between his observations and those of Bary and Stuckey. It is possible that the latter's figure was optically reversed, or that their strain from the southern hemisphere differed from northern hemisphere strains in this respect.

The cells are capable of very sudden and rapid "darting" movements, apparently achieved by a single, strong posterior sweep of the bristlelike cirri. It is not known if the ECB cilia also participate in this rapid movement. At rest the cirri are held out stiffly from the cell body, characteristically in three planes at angles of approximately 50–70° to each other. They arise immediately below the ECB and alternate with the rows of cilia. In living cells it appears that each interciliary site gives rise to only one cirrus. There are no cilia on the cell surface other than those of the ECB and those united into cirri. Several early authors appear to have missed observing the cirri but their observations were usually based on preserved material. With the exception of Parducz's fixative (Parducz 1966) most fixatives used for light microscopy do not preserve the cirri well. In the commercial formalin traditionally employed in the routine preservation of field samples the cells usually disintegrate. However, Fenchel (1968b) apparently found formalin sufficiently useful for counting the cells. In Lugol's Iodine (Utermöhl 1958) the cells remain intact but the cilia and cirri are seriously disorganised (see Bakker 1966, 1967*a*). Bary and Stuckey (1950) observed a strong phototactic response, the ciliate concentrating in a light gradient in the region of moderate illumination. We have observed, during attempts to select individuals with a micropipette, that the cells show a marked negative reaction to local currents, actively darting away from them.

The cells are small for ciliates, 10–70 μ in length (usually 30–50 μ in blooms, and 15–25 μ in winter) and much the same in maximum diameter. As indicated previously, cell shape is highly variable. The cells may be subspherical, lenticular, spindle-shaped or almost bispherical in side view. Although the anterior pole is usually bluntly conical the posterior pole is often flattened and sometimes bears a shallow central depression. The pellicle is extremely thin over most of the cell. Only in the ECB region does there appear to be a rigidity of surface structure.

Within the peripheral region of all the cells we have seen, there are numerous reddish brown bodies, usually 4–7 μ in size, shown to be chloroplast structures (Taylor et al. 1969). In living cells the only other obvious internal feature is the highly vacuolated nature of the endoplasm.

Very little is known of the life cycle of this species, observations on cell division (of particular importance in understanding the nature of the contained chloroplasts) being completely lacking. We have not observed it even though we have maintained cells for a month in incubators, but at the time no serious search was made for division stages. The absence of observations on division suggests a possibility of reproductive synchrony, with division occurring during a dark cycle. Buchanan (MS 1966) has illustrated a specimen of *Mesodinium* (labelled *Cyclotrichium* sp. in his text) in which the posterior pole was enclosed by a loricalike structure (reproduced here as Fig. 6).

This may be an artifact but we doubt this in view of the constriction of the lorica about the narrower part of the cell and the lack of resemblance of the lorica to those of any other species we know of.

Blooms of *M. rubrum* appear as discoloured patches similar in size and shape to those observed in red-water occurrences caused by other organisms such as dinoflagellates. The patches are characteristically much deeper maroon in colour than is usually observed in dinoflagellate blooms. McAlice (1968) has determined the general relation of the intensity of discoloration with cell numbers, the discoloration being undetectable at 200 cells per ml and strong at 2000 per ml.

Although true culture has not been possible as yet, the presence of such vast quantities of the organism has permitted a few authors to study some gross physiological properties of the ciliate. They have found that the cells contain large quantities of chlorophyll *a*, plus lesser amounts of chlorophyll *c*, α-carotene, a phycoerythrin with a unimodal absorption peak at 542 nm, are capable of the fixation of ^{14}C, uptake of ^{32}P, and evolve oxygen (Fonds and Eisma 1967; Bakker 1967a, b; Ryther 1967; Parsons and Blackbourn 1968; and Barber et al. 1969). Furthermore, Ryther (1967) found very low quantities of phaeophytin in relation to the chlorophyll content, suggesting a minimal digestion of the contained chlorophyll.

Distribution

In Table 1 we have listed the publications that refer directly to occurrences of *M. rubrum* (either as such or under an assumed synonym). The numbers on the chart (Fig. 1) key to the references in Table 1. Two of the instances included in Fig. 2 have only been published in manuscript form (Bursa 1961b; Buchanan MS 1966). Geographically the distribution is extremely wide, extending from the polar waters of the Davis Strait and the Kara–Barents Sea area through the equatorial water off Ecuador to the temperate southern hemisphere localities of New Zealand, Peru, and South Africa.

The great majority of records are from extremely neritic locations such as bays, inlets, and fjords. The farthest offshore records are those of Gran (1929a), Grøntved (1952), and Braarud et al. (1953) from the Davis Strait (former) and the North Sea (latter two records). However, in these instances maximum numbers were still found nearer the coast. The largest numbers found away from the coast are those where blooms have been associated with the margins of upwelled water. Blooms were found between 60 and 100 miles off the coasts of Peru and Ecuador by Ryther (1967) and Barber et al. (1969) in the region of transition between the Peru coastal current and the oligotrophic waters further offshore. In the former instance one patch of discolored water was estimated as being 100 square miles in area. A *M. rubrum* bloom has also been associated with upwelling off the coast of Holland (Fonds and Eisma 1967) but in this instance the maximum distance away from the coast was only 6 miles.

Most studies have not provided information on the vertical distribution of the species. Where sufficient data have been given it usually indicates a presence in small numbers to depths of approximately 100 m (e.g., Leegard 1920) but highest numbers are always near the surface. From Bary and Stuckey's (1950) observations on the phototactic response of the ciliate described earlier, it is not surprising that maxima are often found just below, rather than right at the surface during the day unless under slightly overcast conditions such as those described by McAlice (1968). Undoubtedly vertical migration must be taken into account when studying red water caused by this species.

Blooms have been recorded at all times of the year, including winter. However, red water produced by this organism occurs predominantly in spring and autumn with the exception of equatorial regions where no pattern is discernable. In temperate and cold coastal waters the species has been recorded as being present in virtually every month of the year (e.g., Bursa 1961b; Buchanan MS 1966; Bakker 1966; and many Scandinavian studies).

The temperature/salinity/plankton diagram (Fig. 2) shows the exceptionally wide tolerance by this species although *maximum* numbers are clearly related with temperatures close to, but mainly above, 15 C irrespective of salinity. The "hole" in the left centre part of the diagram is not considered particularly significant in view of the rarity of water masses with these intermediate characteristics persistent at or near the surface. It should be stressed that although this species can apparently tolerate a very wide range of temperature and salinity when exposed to *slow* changes, we have seen that it is more susceptible to *rapid* change than most plankton organisms we have studied, probably due to the delicacy of the ciliate's pellicle and the great vacuolar development within the cytoplasm. If *M. pulex* is in fact conspecific with this species, as asserted by Bakker 1966 (who however excludes *C. meunieri* on the basis of Bary and Stuckey's (1950) figures), then *M. rubrum* also occurs widely in fresh water (e.g., Penard 1922; Noland 1937). If *M. pupula* is also conspecific with *M. rubrum*, then the species is common in the reducing zones of marine sediments (Fenchel

TABLE 1. Summarised records of occurrences of *Mesodinium rubrum*.

Ref. no.	Locality	Date of occurrence	Max concn/liter	Name used	Source	Comments
			Authentic records			
1	Point Barrow, Alaska	Summer 1954	–	*M. rubrum*	Bursa 1963	Listed only.
2	Strait of Georgia	Apr. 1933	–	*M. rubrum*	Clemens 1935	Red water. Followed 2 weeks of calms. Assumed feeding by shellfish. Other brief records from this area in Wailes (1943), Herlinvaux et al. (MS 1966), and Quayle (1969). The *Mesodinium* sp. of Eddy (1925) may also be *M. rubrum*.
3	Indian Arm, Vancouver	Sept. 1962 to Aug. 1963	4×10^5	*C. meunieri*	Buchanan 1966	Ph.D. thesis. Recognised two forms similar to Leegard's (1920). Present every month; maximum in August. Temperature and salinity data not in thesis but given to the authors.
4	Departure Bay, Vancouver Is.	Aug. 1967	2×10^6	*M. rubrum*	Parsons & Blackbourn 1968	Red water. Pigment analysis. Bloom occurred after offshore winds.
5	English Bay, Vancouver	June 1969	–	*M. rubrum*	Taylor et al. 1969	Red water. Ultrastructure of chloroplasts.
6	Mission Bay, Calif.	Summer 1961	–	*M. rubrum*	Lackey & Clendenning 1960; Lackey 1967	Record only; *M. pulex* also recorded.
7	San Diego Bay, Calif.	July, Aug. 1959	5×10^5	*M. rubrum*	Lackey & Clendenning 1965	Red water. *M. pulex* and *C. meunieri* also recorded! See also Lackey (1967).
8	G. of Calif.	Aug. 1959	–	*M. rubrum*	Lackey & Clendenning 1963	Red water. Mention only in paper on dinoflagellate bloom.
9	Ecuador	Feb. 1968	–	*C. meunieri*	Barber et al. 1969	Red water. Pigment analysis. ^{14}C and ^{32}P assimilation, O_2 evolution. 60–90 miles off coast.
10	N Peru	Apr. 1966	–	*C. meunieri*	Ryther 1967	Red water patch, 100 sq. miles, 100 miles off coast in vicinity of upwelled water. Pigments, ^{14}C fixation. Also in spring, 1969 (personal communication).
11	S Chile	Mar. 1835	–	–	Darwin 1839	Red water. Microscopic examination of living cells. See also Hart (1943).
12	Igloolik, Foxe Basin	Sept. 1955 to Sept. 1956	1×10^3	*M. rubrum*	Bursa 1961b	Highest numbers in November, lowest in July–Aug. Data also contained in issued ms.
13	Hudson's Bay	Summer 1953, 1954		*M. rubrum*	Bursa 1961a	Among commonest of the ciliates found.
14	Davis Strait	Aug. 1924	1×10^3	*M. rubrum*	Gran 1929a	Maxima recorded at Disco Bay, Greenland and Exeter Sound, Baffin Is. Cell described as small with few cilia (cirri?).
17	Great South Bay, Long Is.	July, Aug. 1961	–	*M. rubrum*	Lackey 1963	Red water. Relative numbers given. Cytology. Sixty-five occurrences. *C. meunieri*

						Baffin Is. Cell described as small with few cilia (cirri?).
						Red water. Related to numbers of pelagic Cytology.
17	Great South Bay, Long Is.	July, Aug. 1961	–	*M. rubrum*	Lackey 1963	Sixty-five occurrences. *C. meunieri* also recorded (in text but omitted from table).
18	Waccasassa estuary, G. of Mexico	–	–	*M. rubrum*	Lackey 1967	Listed in table.
19	Seydis Fjord, Iceland	July 1904	–	*M. pulex*	Paulsen 1909	Red water. Said to be probably the same organism as "*Halteria rubra* Lohmann." See also Paulsen (1934).
20	Trangisvaag Fjord, Faroe Is.	May, June 1913	6×10^{2}	*Mesodinium* sp.	Paulsen 1918	Listed as "rare" but also "constantly present."
21	Plymouth, U.K.	Aug. 1962	2.5×10^{2}	*M. rubrum*	Lackey & Lackey 1963	In small numbers in the plankton. *M. pulex* and *M. cinctum* also recorded from sand and mud flats.
22	Dutch coast	Aug. 1965	2×10^{6}	*M. pulex* "*rubrum*"	Fonds & Eisma 1967	Red water close to shore in patches associated with wind-induced upwelled water. Chlorophyll *a* extraction.
23	Oosterschelde and Veerse Meer	Regularly	–	*M. pulex*	Bakker 1966	Red water. Temperature and salinity data. Detailed discussion of the biology of the ciliate.
24	Oosterschelde and Veerse Meer	May 1965	1.6×10^{7}	*M. pulex*	Bakker 1967a, b	Red water. Chlorophyll, O_2, *p*H, temperature, salinity. See also Vaas (1968) – correlation with lack of turbulence.
25	Neuwerk Is., Elbe estuary	–	–	*M. pulex*	Kahl 1930	In brackish water. Considered "symbiont" was blue-green alga. See also Kahl (1933).
26	Kiel Bay, Baltic	Aug. 1905 to Aug. 1906	3.9×10^{3}	*Halteria rubra*	Lohmann 1908	Original description. Names "symbionts" as *Erythromonas haltericola*. See also Apstein (1908) and Hamburger & Buddenbrock (1911)
27	Danish fjords	May to Sept. 1954	1.8×10^{4}	*M. rubrum*	Grøntved & Nielsen 1957	Comparison between Veno Bugt (higher salinity) and two lower salinity fjords.
28	Isefjord, Denmark	July, Aug. 1968	2.5×10^{8}	*M. rubrum*	Fenchel 1968b	Red water. Cells described. Short list of earlier records. Bloom associated with warm, calm conditions.
29	S North Sea	May 1947	4×10^{3}	*M. rubrum*	Grøntved 1952	Scattered over whole area but maxima close to coasts.
30	North Sea	May 1948	5.5×10^{3}	*M. rubrum*	Braarud et al. 1953	Maximum numbers near coasts, at or near the surface.
31	Oslo Fjord	Summer, autumn 1933	2.1×10^{3}	*M. rubrum*	Braarud & Bursa 1939	Maximum in inner polluted region.
32	Bergen, W Norway	Aug. 1941, Apr. 1942	8.5×10^{2}	*M. rubrum*	Braarud & Hope 1952	Low numbers. See also Fagerland (1945).
33	Julsund, SW Norway	Mar., Apr. 1926	1.2×10^{3}	*M. rubrum*	Gran 1929b	Scattered throughout all material. Maximum near mouth.
34	Central and NW coast of Norway	Mar., Apr. 1945, May 1946	1.2×10^{2}	*M. rubrum*	Braarud et al. 1958	Low numbers. Listed in tables only.
35	Lofoten, N Norway	?	4×10^{3}	*M. rubrum*	Føyn 1929	Reference not seen — derived from Gaarder (1932).

(*Continued*)

TABLE 1. Summarised records of occurrences of *Mesodinium rubrum*. — (*Concluded*)

Ref. no.	Locality	Date of occurrence	Max concn/liter	Name used	Source	Comments
36	Tromsø, N Norway	Summer 1930	5.7×10^3	*M. rubrum*	Gaarder 1932	"...fairly common, occasionally occurring in abundance."
37	Kara/Barents Sea	June–Sept. 1907	–	*Cyclotrichium* sp.	Meunier 1910	Precise locality not given. No ecological data.
38	G. of Bothnia, Baltic	June	2×10^3	*M. rubrum*	Michanek 1965	Most common zooplankton species.
39	G. of Bothnia, G. of Finland	May 1912	4×10^3	*M. rubrum*	Leegard 1920	Recognised two forms, the smaller being most abundant. Occurred in fair numbers to 80 m.
40	Pojo Bay, Finland	July 1936 to May 1937	1.4×10^4	*M. pulex*	Halme 1958	Numbers, temperature, salinity. Recognised Leegard's two forms.
41	Black Sea	?	?	?	Skolka & Petran 1960	Reference not seen — derived from Fenchel (1968b). Reports several blooms in late summer and early autumn.
42	Angolan coast	Oct. 1950	1.3×10^7	*C. meunieri*	Hart & Currie 1960	Red water. Noted probable synonymy with *M. rubrum*. Numerous free chloroplasts ("platelets") from exploded cells.
43	Table Bay, S Africa	June 1930	–	*M. rubrum*	Hart 1934	Red water. Mention only.
44	False Bay, S Africa	July 1934	–	*M. rubrum*	Hart 1934	Red water. Description of the organism. See also comment by Paulsen (1934) and Hart (1943).
45	Wellington Harbour, N.Z.	June, Aug. 1948	–	*C. meunieri*	Bary & Stuckey 1950	Red water. The most detailed cytological description prior to ultrastructural study.
46	Cook Strait coast of South Is., N.Z.	Dec. 1948 intermittently to July 1949	–	*C. meunieri*	Bary 1953	Four blooms confirmed. Several others possible but only seen from the air. Discussed concentrating mechanisms. See also Bary (1951).
			Records possibly attributable to M. rubrum			
47	G. of Mexico	June 1952	–	*M. pulex*	Slobodkin 1953	Red water ..."possibly *Mesodinium pulex* Noland" [sic] "or a closely related form." Mentioned in connection with other red water caused by dinoflagellates.
48	Clyde, U.K.	–	–	*Mesodinium*	Marshall in Michanek 1965	Brief reference in the discussion following Michanek's paper.
49	Caspian Sea	–	–	*M. pulex f. rubrum*	Agamaliev 1967	Listed as a mesopsammic species. Possibly is *M pulex*. Not abundant.
50	Ria de Vigo, Spain	Aug. 1955	1×10^6	*M. pulex*	Margalef 1956	High O_2 production. Mechanism for concentration discussed. Tentative identification apparently influenced by the case of Slobodkin (1953).

1969). The wide aerobic marine distribution accepted for *M. rubrum* by us here is not exceptional in view of the highly cosmopolitan distribution exhibited by a great many marine psammophilic ciliates (Fauré-Fremiet 1951; Raikov and Kovaleva 1969).

Detailed Structural Observations

Materials and Methods

All observations here were made on unconcentrated seawater samples containing appreciable quantities of the organism. Most of the specimens examined resulted from the sampling of red water occurring in English Bay, Vancouver, B.C., in June 1969 (temperature 17 C, salinity 8.5‰). In fluorescent light-equipped incubators the cells survived for periods of up to 1 month. Little difference was noted in survival between temperatures of 10 and 20 C.

Ten ml of the cells in sea water were gently fixed for 4 hr at 4 C in a solution containing 2% glutaraldehyde, 0.1 M cacodylate buffer, and 1% sucrose with a resultant *p*H of 7.3. The cells were then washed twice in the buffer/sucrose solution above, followed by postfixation, again at 4 C, in the same buffer/sucrose solution with 1% osmium tetroxide for 15 min. This was followed by dehydration through a graded series of cold alcohols. The cells were embedded in Epon and cut on a Porter-Blum MT-2 ultramicrotome. Sections were double-stained with uranyl acetate and lead citrate and examined with a Philips 200 electron microscope.

Less satisfactory fixations involved a procedure similar to the above but in one instance glutaraldehyde fixation was carried out for only ½ hr at room temperature, and in another instance the prefixation with glutaraldehyde was omitted. Initially cells were treated with the "Parducz Instantaneous Fixation and Staining Technique" (Parducz 1966) for ciliates, but this was adequate only for observation of gross ultrastructural features and was extensively used for light microscopy.

The Cilia

The cilia of the ECB forming the corona arise in perpendicularly arranged rows (kineties) consisting of double rows of approximately 15 kinetosomes in each and in an alternate arrangement with one another (Fig. 7). They do not usually insert perpendicularly to the surface but instead arise from the right edge of ridges (Fig. 20, 22).

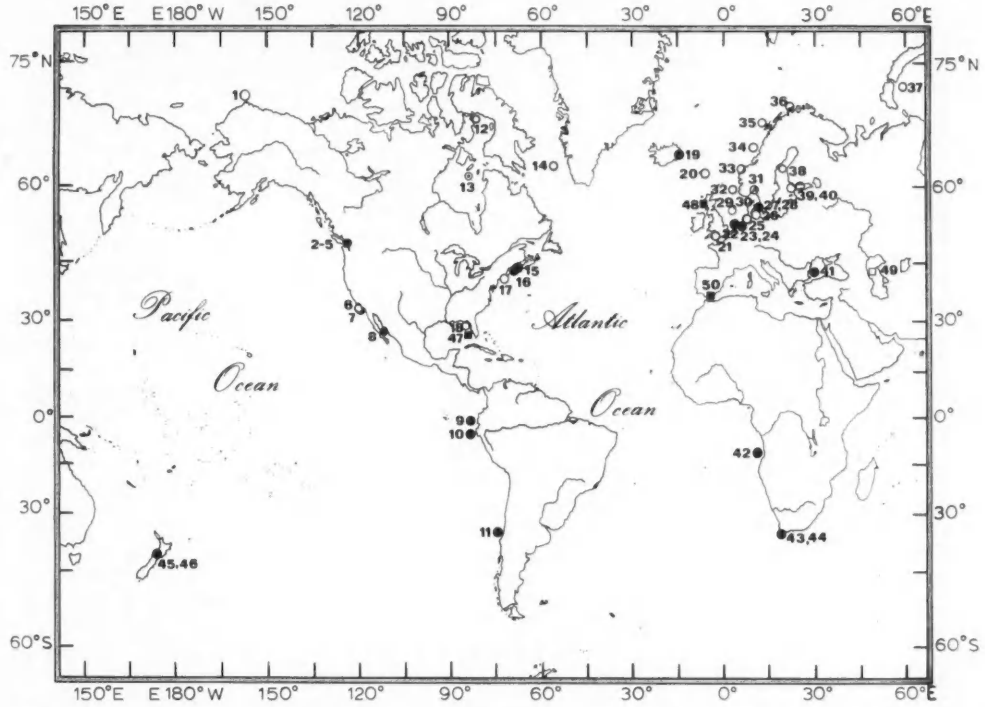


FIG. 1. World distribution of *Mesodinium rubrum*. Numbers key to the records in Table 1. Circles indicate probable records; squares indicate more dubious records. Red water "blooms" are shown by solid symbols.

The only other cilia are those grouped together to form the cirri, stiffly extended in three planes (Fig. 3–5) in the living organism. The cirri appear to arise singly at the cell surface, immediately posterior to the ECB. With the electron microscope we found groups of 16 kinetosomes lying immediately posterior to and alternate with the ECB kineties (Fig. 7, 16). Thus it might be assumed that each cirrus is composed of a bundle of 16 cilia. This does not, however, appear to be the case. In fixed cells the extended cirri cannot be seen. Instead bundles of eight cilia lie close to the posterior cell surface in the vicinity of shallow, asymmetrically shaped depressions (Fig. 18). It seems reasonable to assume that these octets are the cirri in a "closed beat" position, presumably a response to fixation. We are then, however, left with eight of the 16 post-ECB kinetosomes unaccounted for. There seem to be two possible explanations for this puzzle. Firstly, each post-ECB cluster of kinetosomes may give rise to two cirri, each composed of eight cilia. One series of these may lie so close to the cell body that they are not seen by the light microscope. Secondly, eight of the kinetosomes may not produce cilia. Bary and Stuckey (1950, fig. 2) figure a fixed, sectioned cell in which extended cirri are absent and only posteriorly directed clusters of cilia (presumably our "octets") are present. However, Bakker (1967a, fig. 1*a*) has illustrated an appearance that may support the first hypothesis (his figure is inverted, showing the "oral" end uppermost).

In addition to these functional groupings of cilia we have also observed two types of ciliary

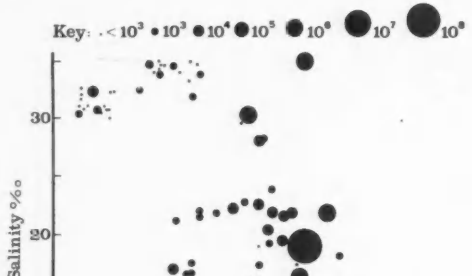


FIG. 2. Relation of numbers of *M. rubrum*/liter (size of circle) with temperature and salinity. Data compiled from references in Table 1. Most of the points to the right of 10 C are from the fjord localities of Scandinavia and British Columbia. The extreme top left values are from Arctic waters, and the low salinity values at bottom left are Baltic Sea records.

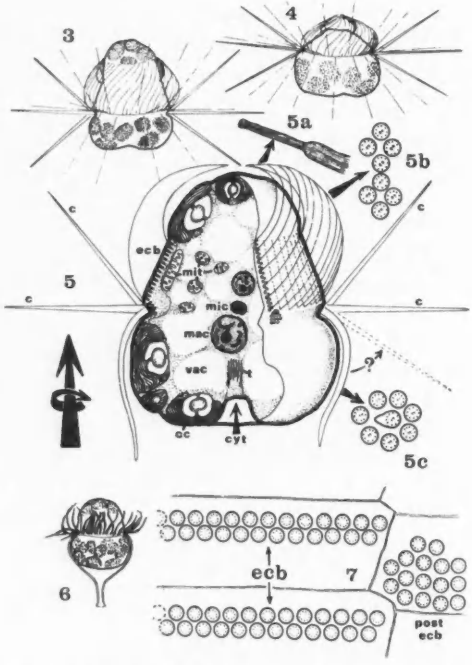


FIG. 3, 4. Sketches indicating extremes of morphological variability in living cells, chiefly the result of variable development of the aboral end (uppermost). Figure 4 may represent an individual immediately after fission. FIG. 5. Main structural features of *M. rubrum*. Organism's right side cut away. Large arrow at left indicates direction of movement. (*a*) Detail of distal portions of ECB cilia, showing terminal narrowing containing central microtubules only. (*b*) Functional grouping of the distal parts of the ECB cilia. (*c*) Post-ECB ciliary octet. Not known if these correspond to extended cirri. FIG. 6. Lugol's Iodine-preserved individual with "lorica." See text. Redrawn from Buchanan (MS 1966). FIG. 7. Diagrammatic representation of kinetosome arrangement, turned sideways, anterior to left, posterior to right. Post-ECB asymmetry precise and constant (2,3,4,4,3, in rows). *c*: cirri; *cc*: chloroplast complex; *cyt*: rudimentary cytostome; *ecb*: equatorial ciliary band; *mac*: macronucleus; *mic*: mironucleus; *mit*: mitochondria; *t*: "trichocysts"; *vac*: vacuole.

differentiation. The distal ends of the corona (ECB) cilia narrow abruptly to one-third of their diameter (from 0.47 to 0.15 μm) for a distance of approximately 2.5 μm (Fig. 5*a*). The nine peripheral microtubular duplets are absent from these terminal portions, although the central pair of microtubules continue to the end. The second type of differentiation can be observed in Fig. 18 in which the central member of each of the bundles of eight posteriorly directed cilia contains asymmetrically distributed packing material. This is a constant feature of the ciliary octets. It can also be seen in a short portion of longitudinally sectioned cilium in Fig. 19.

The Cortex and Infraciliature

A distinct membrane-delimited cortex is most evident in the region where the cilia arise and is thicker beneath the post-ECB ciliary bases than beneath the ECB. Elsewhere the cortex is poorly defined and very thin. The ciliate is enclosed over most of its surface by a few membranes, the precise number of which is difficult to determine due to folding (Fig. 15). The pellicular region is very thin, and alveoli are completely lacking. This is a highly unusual condition for a ciliate (see Pitelka 1969, 1970).

The kinetosomes of the ECB kineties penetrate approximately two-thirds of the cortex. The only distinct fibrillar system in this region consists of the strongly developed bundles of fibrils (Fig. 21, 24) arising from the interstices between the kinetosomes on one side only (Fig. 24). The bundles are distinctly wound in a spiral.

The infraciliature beneath the post-ECB cilia has not been completely determined. There appear to be four components involved. A short distance below the surface the groups of 16 ciliary bases are knit together and to adjacent groups by a meshwork of fibrils constructed in a triradiate pattern (Fig. 16) lying parallel to the cell surface. A lateral supportive function for this triradiate meshwork seems likely. Proceeding downwards into the cell from the ciliary bases are "primary fibrils" that meet in plexi, which in turn are connected by "secondary fibrils" to a system of even deeper plexi, the latter being laterally connected to each other by additional fibrils (Fig. 19, 23).

An unusual feature was the presence of striated fibrillar structures not directly attached to the kinetosomes. These occurred in a single row, one beneath each group of 16 post-ECB ciliary bases and connected to the triradiate meshwork of the latter (Fig. 16), also exhibiting deeper fibrillar connections (Fig. 17). They appear to function as anchors to the whole fibrillar complex of the post-ECB cilia.

Another feature worthy of special mention was the observation of pairs of kinetosomes lying deeper in the ectoplasm in the close vicinity of, and oriented at approximately right angles to the kineties of the ECB (Fig. 20). These do not apparently give rise to cilia, and are reminiscent of the diplosome arrangement of centrioles in the Metazoa. These may be duplicated kinetosomes, but they do not correspond to the ultrastructural observations of Allen (1969) and Kalnins and Porter (1969) on the morphogenesis of kinetosomes. However, an arrangement of this type has been reported from gastrodermal cells of *Hydra* by D. Fawcett (cited in Threadgold 1967).

Parasomal sacs were not clearly evident close to the kinetosomes. In Fig. 24 we have labelled some structures that may be parasomal sacs, although membranes delimiting their inner surfaces could not be seen.

Mucocysts (Fig. 25, 26) similar to those described from *Didinium nasutum* O. F. Mueller (Yagui and Shigenaka 1965 (termed "ectoplasmic vacuoles") and Wessenburg and Antipa 1968) are present but are somewhat less frequent and less regularly arranged. They appear to be most abundant in the ECB region. We have been able to resolve the paracrystalline inclusions more clearly than the above authors. The inclusions consist of hexagonally packed granular rods of unequal length, the whole paracrystalline core showing the hexagonal packing in shape. Each is surrounded by a distinct electron-transparent zone.

Large microtubules arranged in parallel arrays can also be seen in Fig. 25 and 26. In view of their alignment perpendicular to the cell surface they may correspond to the "primary fibrils" of *D. nasutum* (Yagui and Shigenaka 1965). In addition to these there are other microtubules that run parallel to the cell surface (Fig. 15) within the outer membrane layers.

The Endoplasm and its Organelles

One of the most striking features of the ciliate is the highly vacuolated state of the endoplasm (Fig. 5, 8, 9). The vacuoles are not restricted to one end of the cell, as in the case of *M. pupula* (Dragesco 1963). With few exceptions the vacuoles appear to be devoid of solid contents. We think that they may play an important role in the remarkable adaptability of the species to wide temperature and salinity ranges.

Mitochondria of the usual ciliate type are scattered throughout the endoplasm with a marked concen-

tration in the vicinity of the ECB. Long mitochondria closely underlie the ECB kineties. In agreement with Bary and Stuckey (1950) we have found a maximum of four ovoid to spherical macronuclei and only one micronucleus per cell. Frequently one pair of the macronuclei are similar in eu- and hetero-chromatin distribution, the other two being in a different state but also similar to each other. This suggests the possibility that with regard to DNA synthesis the macronuclei may be linked in pairs (see review by Raikov 1969; also Milner 1969).

A rudimentary cytostome has been observed in two cells (one figured in Fig. 9, 10). A nemodesma (Fauré-Fremiet 1961; Pitelka 1969) of the type used by predatory holotrichs such as *Didinium* is not evident in sections of the cytostome. A cluster of rodlike bodies of the type referred to as trichites in earlier ciliate literature can be seen in the cytostomal cytoplasm (Fig. 10). We have not yet been able to determine if they are "pexicysts" or "toxicysts" as described from another didiniid, *Didinium nasutum* (Wessenburg and Antipa 1968).

The presence of a cytostome at the pole posterior to the direction of locomotion is unusual for the Didiniidae and may be interpreted in terms of homologies to infer that the cells in fact move "backwards." Several authors (e.g., Bakker 1966; Fenchel 1968a, b), presumably on this basis, figure the cytostomal end uppermost. Throughout this paper we have designated the orientation according to the direction of locomotion. According to Noland (1937) *M. pulex* also moves backwards.

The Chloroplast/Mitochondrial Complexes

A distinctive feature of the ciliate, as indicated in a preliminary communication (Taylor et al. 1969), is the presence of many similar, algal chloroplasts in the peripheral cytoplasm.

The arrangement of the complexes within the ciliate is relatively ordered. They are always strongly peripheral, absent from beneath the ECB kineties, with the flattened chloroplast outermost (see Fig. 13–15). A distinctly stalked pyrenoid projects from the inner surface of each chloroplast. A pair of hemispherical "lenses" of starch surrounds the pyrenoid. Also associated with the complexes are mitochondria containing thin flattened cristae, noticeably distinct from the numerous mitochondria of the ciliate with their tubular cristae. The only structures observed other than these and the membrane systems detailed below are a coarsely granular (ribosomal) matrix in which the mitochondria lie, and osmiophilic droplets. Most unusually, nuclei have not as yet been observed in association with the complexes.

At least three, possibly four, concentric double membrane systems have been observed in the complexes. The innermost of these is the chloroplast membrane that also closely invests the pyrenoid. A second invests the chloroplast membrane, and also the starch hemispheres, with very little enclosed cytoplasm. Between this second and a third investing membrane pair lie the coarsely ribosomal cytoplasm and the nonciliate mitochondria. The space between the components of the second and third membrane pairs varies widely in places, suggesting that they may be derived from an endoplasmic reticulum system. A fourth membrane pair can sometimes be observed close to the third. Beyond this it is difficult to discern further investing membranes, if any.

The gross features described above agree with the light-microscope observations of Powers (1932), Bary and Stuckey (1950), Fonds and Eisma (1967), and Fenchel (1968a, b). Further, Dr S. Watson of the Woods Hole Oceanographic Institution has kindly provided us with some electron micrographs of sectioned material collected from red water off Peru in April 1966 (Ryther 1967) and spring 1969. Although severely subjected to osmotic swelling the features of the chloroplast/mitochondrial complexes agree closely with the British Columbia specimens but have less starch surrounding the pyrenoid in all instances.

Taxonomic Difficulties

At present the correct name for this taxon, or even the number of taxa actually included in Table 1, is difficult to resolve in view of the lack of detailed modern descriptions of the taxa other than *M. rubrum* and *M. pupula*. Also, earlier treatments of the taxonomy of the genus were neither thorough nor critical. Some of the confusion may perhaps be cleared away by the following outline and at least the distinction at the generic level between *Mesodinium* Stein and *Cyclotrichium* Meunier made clear.

In 1862 Stein created the genus *Mesodinium* for a taxon formerly known as *Halteria pulex* Claparède et Lachmann. The key characters employed were the lack of general somatic ciliation, an equatorial constriction into dissimilar halves, and long "griffelartige" cirri arising from the constriction. A spiral movement was mentioned. The cytostome was described as anterior. No mention was made of cilia other than those in the cirri. Kahl (1930) amended this description by inclusion of a second whorl of cilia thought to be posterior to the cirri in a left spiral arrangement, noting that the latter could be easily overlooked.

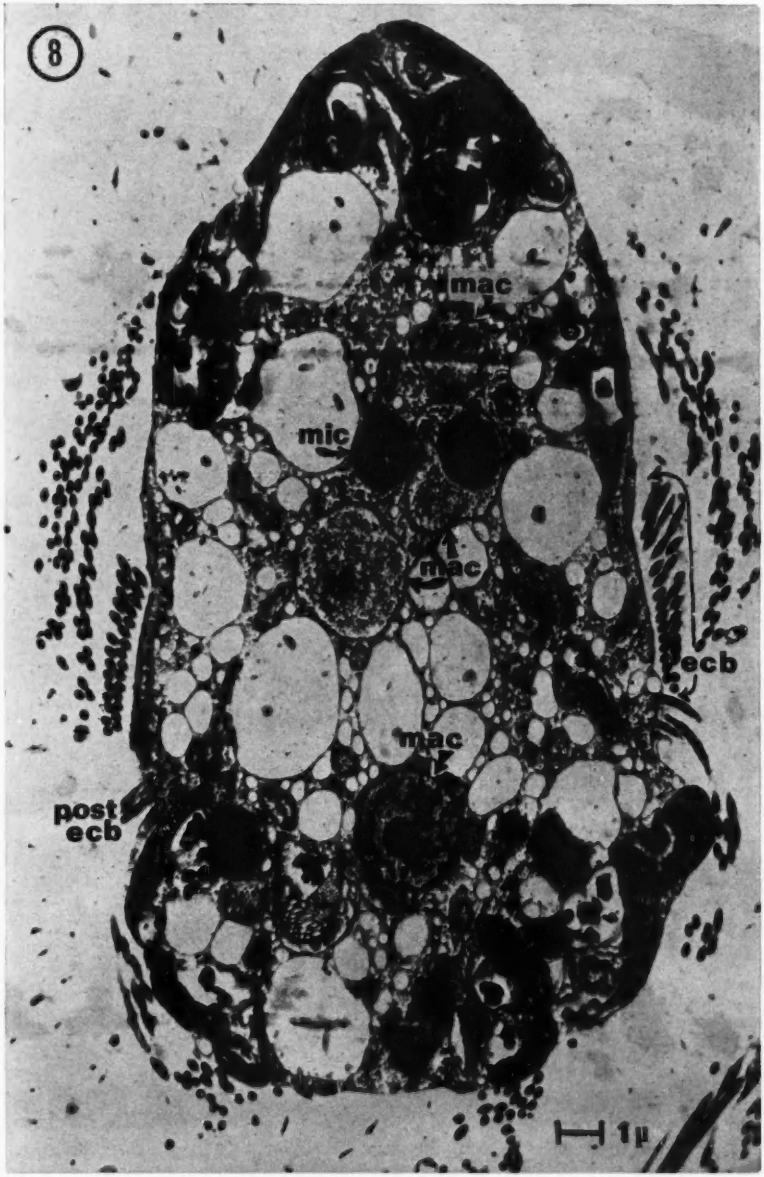


FIG. 8. Near longitudinal section illustrating main features of *M. rubrum*. Buffered osmium tetroxide fixation only, poor, particularly for chloroplast complexes. ×5800. *ecb*: equatorial ciliary band; *mac*: macronuclei; *mic*: micronuclei; *post ecb*: post-equatorial ciliary band cirri.

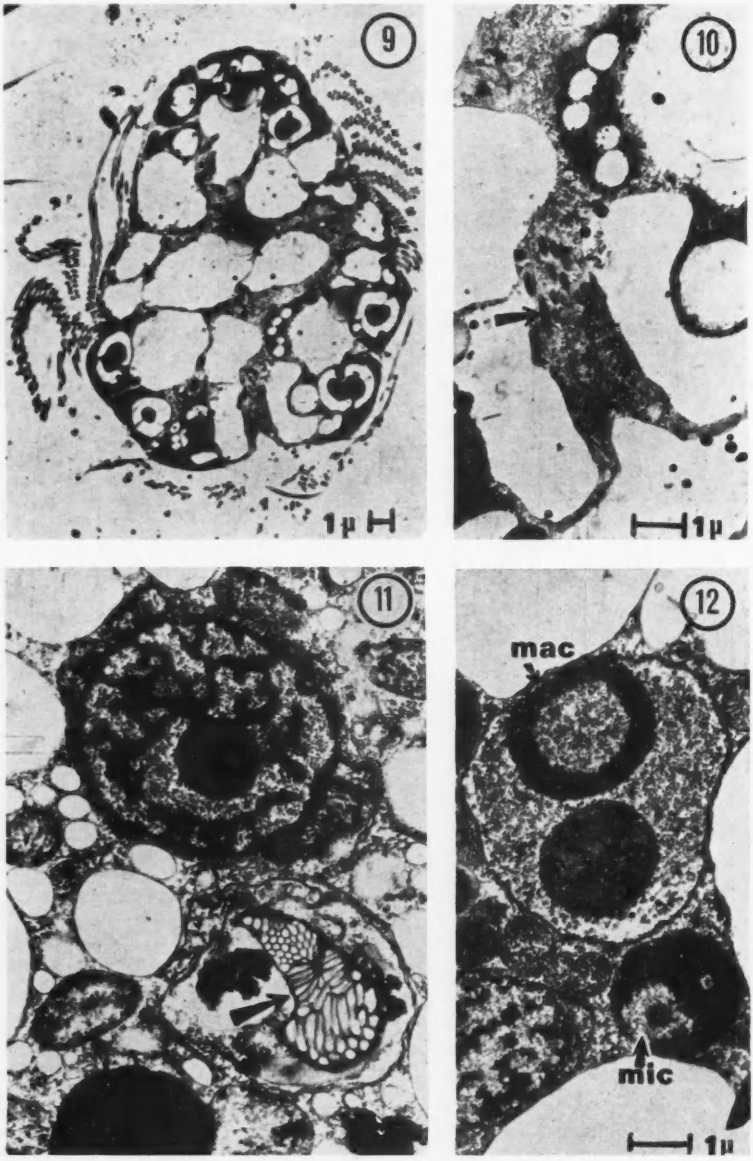


FIG. 9. Near longitudinal section of glutaraldehyde/osmium tetroxide fixed specimen. Note distribution of the chloroplast complexes and the rudimentary cytostome. ×2900.
FIG. 10. Detail of the cytostome in Fig. 9. Arrow indicates the "trichocysts." ×7500.
FIG. 11. Details of a macronucleus from Fig. 8. The nature of the arrowed structure is unknown but it may be the only evidence for digestion of one of the chloroplast complexes (note the mitochondria with flattened cristae within the vesicle and close to the nuclear membrane). ×11,800.
FIG. 12. A macronucleus containing a ring of heterochromatin, and a micronucleus (glutaraldehyde/osmium fixation). ×9100. *mac*: macronucleus; *mic*: micronucleus.

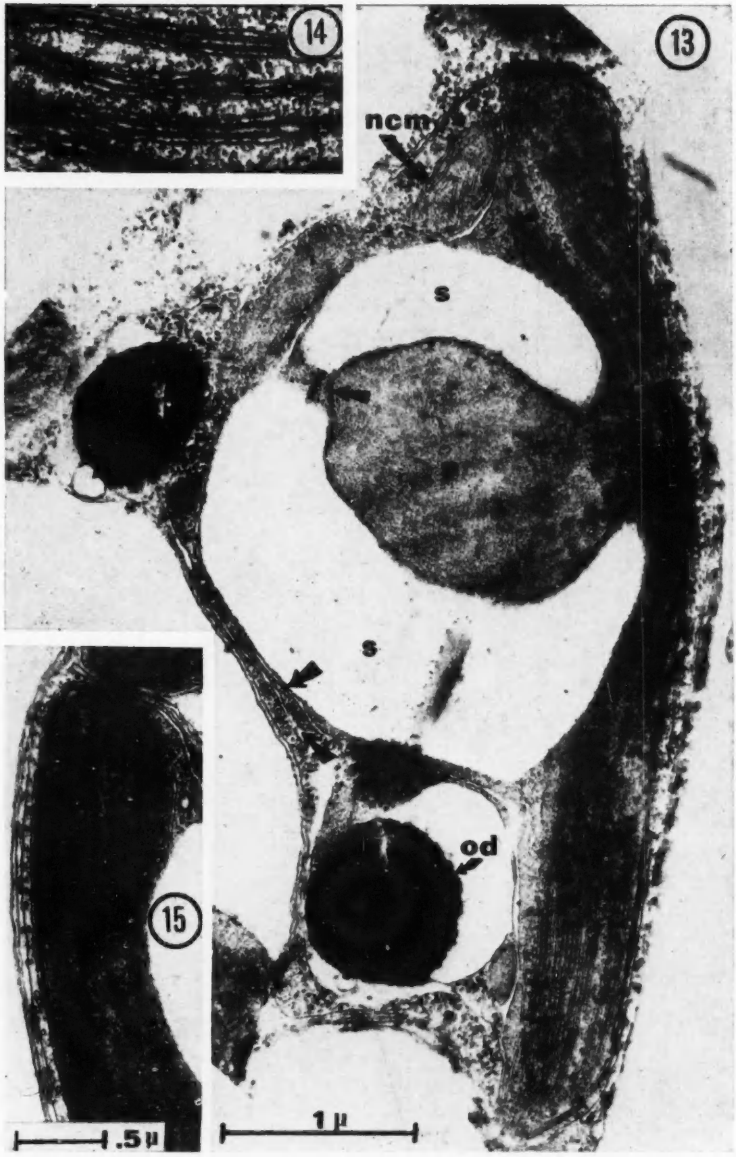


FIG. 13. Longitudinal section through a chloroplast/mitochondrial complex. Unlabelled arrows indicate the enveloping membrane systems. Exterior of the ciliate to the right. ×29,000. *ncm*: nonciliate mitochondria; *od*: osmiophilic droplet; *p*: pyrenoid; *s*: starch.

FIG. 14. Detail of the type of thylakoid grouping observed. ×39,200.

FIG. 15. Detail of the outer membranes of the ciliate in the vicinity of a chloroplast. Note the peripheral microtubules. Exterior of the ciliate to the left. ×26,000.

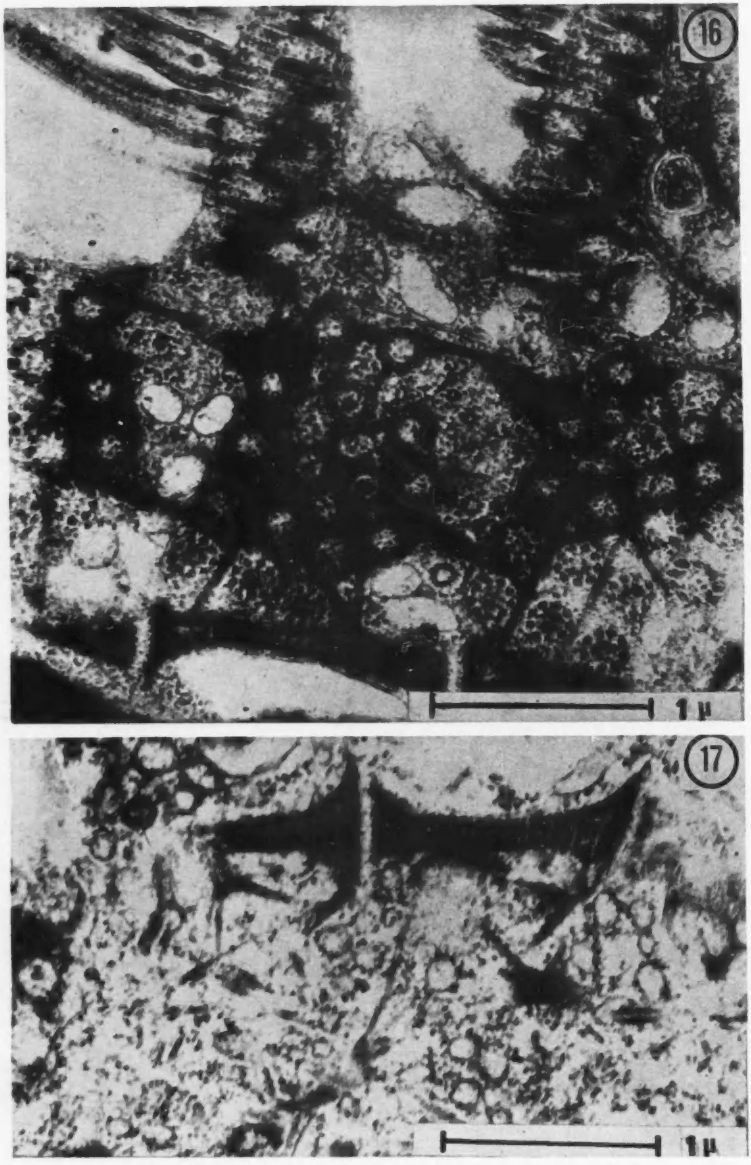


FIG. 16. Oblique section close to the surface at the base of the kineties showing the "webbing" that knits the groups of 16 post-ECB kinetosomes together and the anchoring bands. ×32,600.

FIG. 17. Section of the striated anchoring bands perpendicular to the cell surface showing complex deep, fibrillar interconnections. Exterior of the cell at the top. ×32,600.

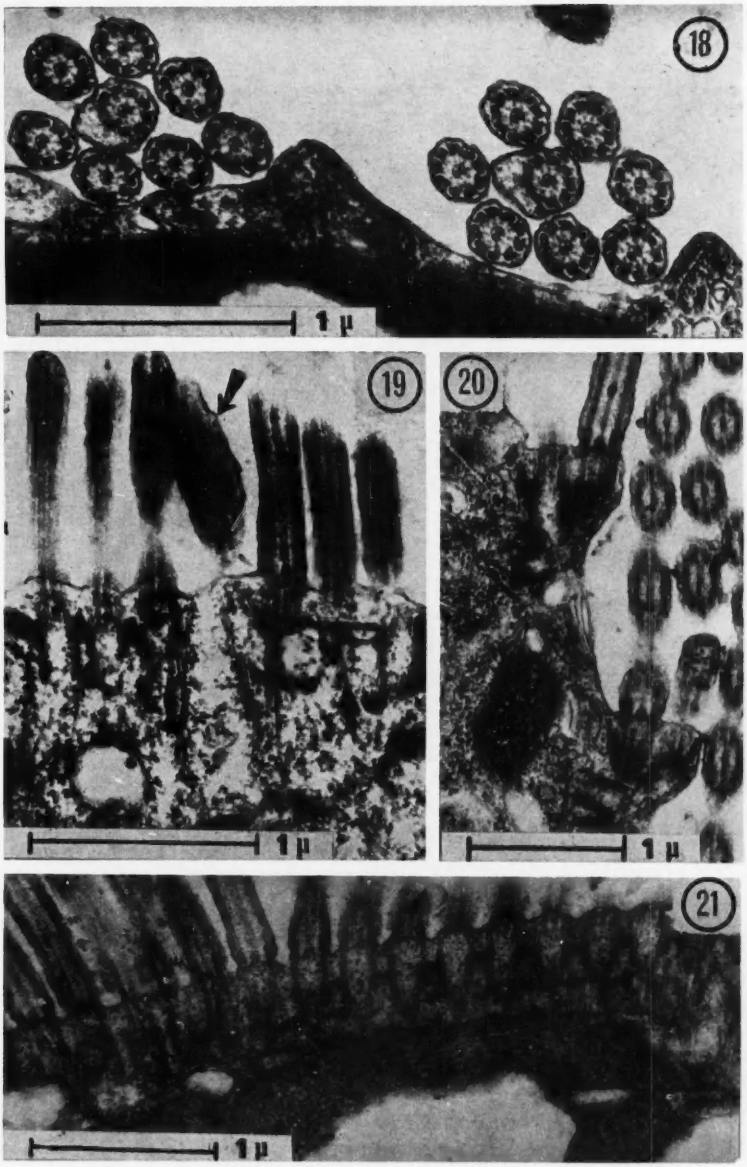
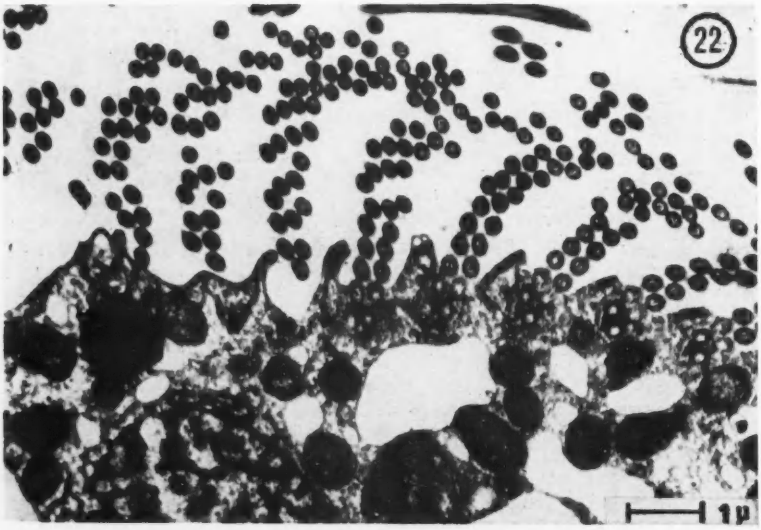


FIG. 18. Cross section of the posterior ciliary octets, presumably cirri. Note the asymmetric packing material of each central member and the asymmetry of the depressions in the surface of the cell with microtubular strengthening concentrated on one side. ×39,200.
FIG. 19. Poor, osmium-fixed section perpendicular to the surface, included here because it fortuitously provides a partial longitudinal section of one of the asymmetrically packed members of the ciliary octets (arrow). ×34,000.
FIG. 20. Cross section through two ECB kineties showing the manner in which the cilia arise in double rows from the right inner surface of ridges on the cell surface. Note also the deep kinetosomes at right angles to those giving rise to cilia. ×23,600.
FIG. 21. Longitudinal section of an ECB kinety in which the cortex is clearly demonstrated and closely underlain by a large elongate mitochondrion. ×28,000.



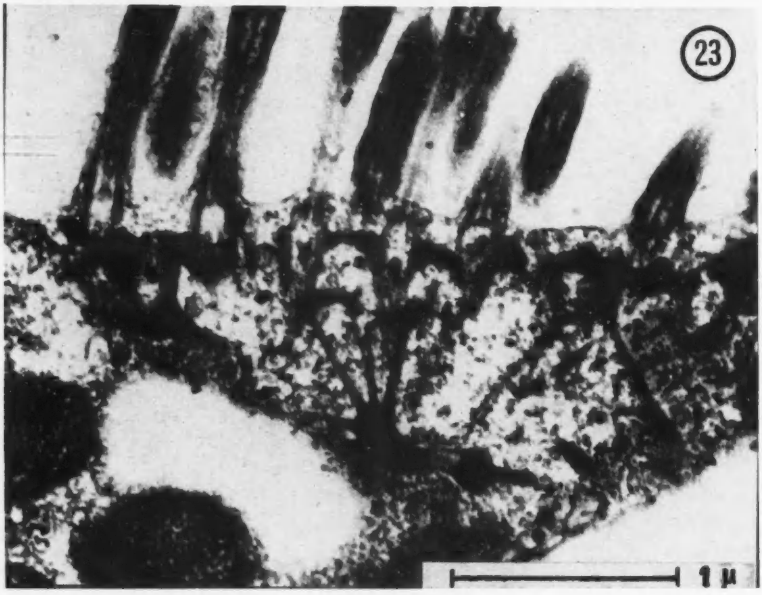


FIG. 22. A near-perpendicular section through the ECB showing the surface folds or ridges and the doubled groups of cilia that comprise the ECB corona. ×11,000.
FIG. 23. Detail of the fibrillar systems and plexi underlying the post-ECB kinetosomes. ×34,900.

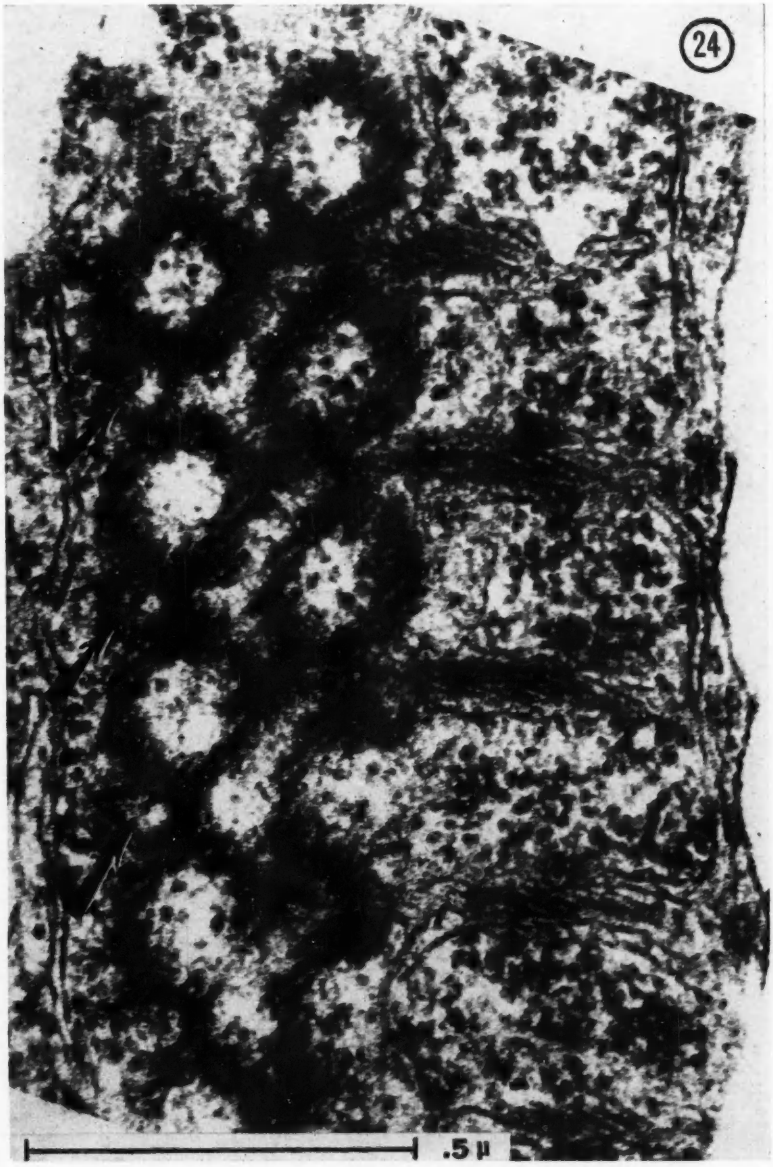


FIG. 24. Oblique section near the surface of a double ECB kinety in which the spirally twisted nature of fibrillar structures, presumably "right radial strands," can be seen arising between adjacent kinetosomes on one side only. Arrows indicate possible parasomal sacs. ×116,500.

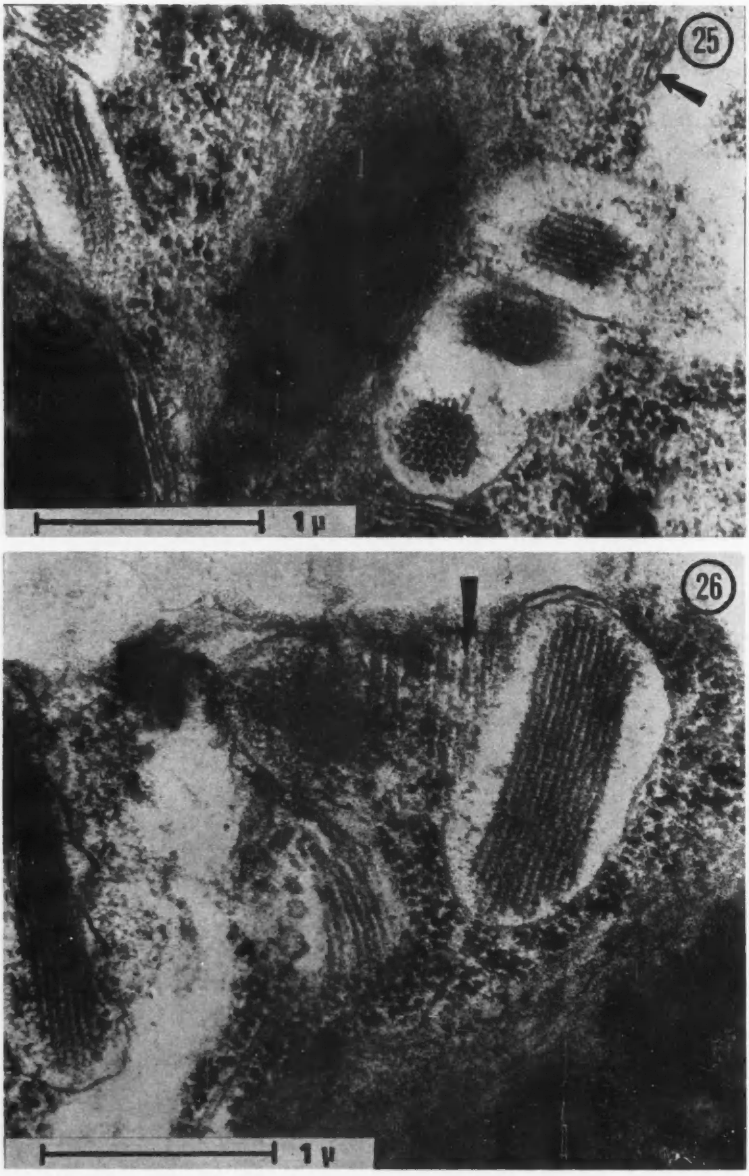


FIG. 25, 26. Two details of mucocysts and their enclosed paracrystalline structures seen in both longitudinal and cross section. These mucocysts lie close to the cell surface within the cortex. Note also the arrays of "primary fibrils" in the cytoplasm between the mucocysts (arrows). Both ×34,300.

The genus now involves the following taxa: *M. pulex* (Clap. et Lachm.) Stein, *M. acarus* Stein, *M. rubrum* (Lohmann) Hamburger et Buddenbrock, and *M. pupula* (Kahl) Dragesco. *Mesodinium cinctum* Calkins has been considered as probably conspecific with *M. pulex* by Kahl (1930), and *M. fimbriatum* Stokes remains enigmatic, not apparently observed by anyone other than the original author. Kahl (1930) considered it to be a synonym of *M. acarus* but it is very large, 225 μm in size with small side branches on the cirri. *Mesodinium phialinum* Maskell was also considered a synonym of *M. acarus* by Kahl. *Mesodinium recurvum* Kellicott should be in the genus *Cyclotrichium* Meunier (see below). Leegard (1920) has recognised two forms of *M. rubrum* related to size: forma *major* and forma *minor*. Various authors have proposed infraspecific status for *M. rubrum* and *M. pupula*, listing them as forms or subspecies of *M. pulex* (see Table 1).

The criteria on which these closely similar taxa may be separated are not at all clear in Kahl's monograph (1930, 1935), and in some instances it is evident that separations have been based on faulty or incomplete observation. For example Noland (1937) has indicated that Kahl's distinction of *M. acarus* from *M. pulex* on the basis of the absence of "membranelles" in the former (ECB corona) was in error. On the other hand Noland provided a more precise means of distinguishing them on the basis of the distal ends of the circumoral "tentacles."

In the absence of detailed information on several of the species one can only arbitrarily select criteria by which the taxa can be separated. A simple key is given below.

1*a*. Circum-oral tentacles present, oral end distinctly conical.....2

1*b*. Circum-oral tentacles absent, oral end rounded...3

2*a*. Distal ends of tentacles capitate.....*M. acarus* Marine and freshwater; cells small, usually less than 16 μm in length. No enclosed chloroplasts reported. Distinctive locomotion, the cirri being used continuously to move in the oral direction. Our observations indicate that the cirri may bifurcate distally in this species.

2*b*. Distal ends trifurcate.....*M. pulex* Marine and freshwater, but chiefly latter. Larger, up to 50 μm in length. Similar in structure to *M. rubrum*. No obvious symbiotic organelles within.

3*a*. Planktonic, with reddish brown chloroplasts, cytoplasm strongly vacuolated....*M. rubrum* As described here.

3*b*. Within marine sediments, particularly in reducing conditions, one vacuole only...*M. pupula* Colorless bodies reported inside by Kahl (1933). Size 20–30 μm, rarely to 50 μm. Can also appear strongly pigmented due to ingested food. Cells less sensitive than *M. rubrum* to disintegration. Cirri often used semicontinuously.

Mesodinium pulex and *M. acarus* have been reported as feeders on bacteria (Webb 1956), whereas *M. pupula* feeds on pigmented flagellates (Dragesco 1963) and may sometimes exhibit a greenish pigmentation (Fenchel 1968a). Buddenbrock (1920) has figured individuals referred to him as *M. pulex* in which the tentacles are used to grasp detrital particles.

There seems little doubt that *Cyclotrichium meunieri* Powers (1932) is the same organism as that referred to by many authors, including us, as *M. rubrum* (a conspecificity also suggested by Kahl 1935; Hart and Currie 1960; and Fenchel 1968b). Reference has already been made to the similarity of the chloroplast complexes as seen under electron microscopy of the Peru material referred by Ryther (1967) to *C. meunieri* and our British Columbia material. However, the electron micrographs of the Peru material do not allow adequate comparison of the ciliate hosts.

Powers (1932) stated that *C. meunieri* was assigned to the genus *Cyclotrichium* Meunier (1910) on the basis of resemblance to some individuals referred to by Meunier (1910) as *Cyclotrichium* sp. The description of the latter by Meunier was very incomplete and, judging from his figures, may have included several different forms. Those figured on his pl. 15, fig. 7 and pl. 20, fig. 17 are probably the present taxon, the appearance resembling that of *M. rubrum* fixed with iodine (see also Bakker 1966, 1967a). One specimen was also recorded by Meunier as showing a starchlike staining reaction.

Bary and Stuckey's (1950) designation of their specimens as *C. meunieri* Powers was determined by the undoubted similarity of their New Zealand specimens with those of Powers (with the exception of the absence of cirri in Powers' description) and a dissimilarity to the figures of Lohmann's *M. rubrum* reproduced by Hamburger and Buddenbrock (1911), Lohmann's original description not being accessible to them. The latter circumstance was unfortunate as Hamburger and Buddenbrock figured only two (original fig. 37, 38) of the three figures provided by Lohmann (1908 pl. 17, fig. 37–39), and it is Lohmann's fig. 39 that is most similar to individuals in a bloom, showing an individual packed with chloroplasts and lacking an obvious cytostome. In the two other figures

there is a strongly developed conical oral end (lacking tentacles) and the cells are colorless, only one ring of cilia being shown. Lohmann (1908) was of the opinion that these were all part of a progressive development of the symbiotic state. This aspect will be considered in the discussion section that follows.

Unquestionably, as the genera are now constituted, the present taxon should not be placed within the genus *Cyclotrichium* Meunier. Meunier, in his original description (1910 p. 164 et seq.), singled out only the one or two circular zones of cilia as a distinctive generic feature, leaving the characteristics of the type species *C. cyclokaryon* Meunier to round out the description. Fauré-Fremiet (1924) described three further species, all of which possess fine meridionally placed rows of cilia over the posterior pole in addition to a single circular belt of long cilia surrounding an anterior buccal area ("aire buccal"). These features were incorporated into the generic description given by Kahl (1930 p. 129). With the exception of six moderately elongate, asymmetrically placed cilia ("rame ciliaire postérieure") in *C. ovatum* Fauré-Fremiet, there are no structures remotely similar to the ring of post-ECB cirri in *Mesodinium*. Powers (1932) apparently missed the latter in his specimens due to their disappearance after fixation. Consequently his original description of *C. meunieri* was not at odds with the concept of *Cyclotrichium* outlined above.

We would like to suggest that the distinctions between the genera *Mesodinium* Stein and *Cyclotrichium* Meunier be strengthened by placing only those taxa with an ECB *plus* a circle of cirri in the former genus. In addition, all described species of *Mesodinium* lack general somatic ciliation. Species of *Cyclotrichium* do not show an equatorial constriction and further, all described to date possess a single circular macronucleus, a feature not known in any species of *Mesodinium*.

One final element of taxonomic confusion is introduced by the possibility that the neglected genus *Megatricha* Perty (1852) may have been based in part on individuals of *Mesodinium* or *Cyclotrichium*. Leegard (1920) has suggested this by including *Megatricha partita* Perty as a possible synonym for *Mesodinium rubrum* (listed, but no further comment given). Curiously we can find no mention of the genus in Kahl's otherwise encyclopediac work (1930–35). This is another instance where the early descriptions are scanty and the figures so poor that it is difficult, if not impossible, to reach a satisfactory decision. In fact *Megatricha partita* might be considered similar to *Mesodinium acarus*, being 16–18 μ in size with an equatorial constriction, pointed anterior section, rounded posterior section, delicate, "swimming often interrupted suddenly by a jerk in another direction" (Pritchard's translation 1861 p. 615). However, in some of the figures somatic ciliature appears to be present. Perhaps it would be best to leave *Megatricha* in the limbo to which it has been relegated.

Discussion

The most enigmatic aspect of *M. rubrum* is the nature of the pigmented bodies within the ciliate and the functional and theoretical evolutionary significance of the condition. Formerly there was no direct evidence for the presence of chloroplasts. From pigment studies, gross morphological resemblance, and functional aspects determined from field experiments, it had been deduced that the pigmented bodies within *M. rubrum* were entire, endosymbiotic cryptomonad cells (Parsons and Blackbourn 1968; Barber et al. 1969). Our study has shown that true chloroplasts are present in association with mitochondria and membrane systems not belonging to the ciliate. However, no nuclei were observed in association with these chloroplast/mitochondrial complexes despite the examination of hundreds of sections with the electron microscope. In this connection Bary and Stuckey (1950) failed to demonstrate the presence of nuclei in the complexes using the Fuelgen technique and we were also unable to do this employing aceto-carmine staining. If nuclei are present in the vicinity of the chloroplasts they must be extremely reduced in size and, presumably, function. In addition, the thylakoid characteristics of the chloroplasts do not match those of any of the cryptomonads studied so far.

It is evident that the pigmented bodies cannot be simply referred to as symbiotic algae, despite the experimental evidence that they are photosynthetically functional (Ryther 1967; Barber et al. 1969). Further, they exhibit a more complex ultrastructural condition than the instances where chloroplasts from ingested food are preferentially retained for several weeks in a functional condition by some opisthobranch molluscs (Kawagati and Yamasu 1965; Taylor 1967, 1968, 1970; Trench 1969; Trench et al. 1969) or by mouse fibroblast cells in tissue culture (Nass 1969).

In explaining the condition in *M. rubrum* the choice seems to fall between two alternatives, which differ principally in time scale. If the association is temporary (less than annual in duration), arising from the ingestion of one or more species of flagellates, followed by partial digestion of the outer parts of the flagellate and the nucleus, then the condition may be interpreted as similar to the

chloroplast maintenance by opisthobranchs mentioned above. This would require that the chloroplasts be replaced from newly ingested cells periodically. The longest period that ingested chloroplasts have been reported to remain functional is 6 weeks (Trench et al. 1969 in the mollusc *Tridachiella diomedea*). Further, if the species of flagellate is always the same, then the distributions of it and *M. rubrum* must be approximately coeval. To allow for vagaries in the latter the association would have to be facultative, and nonpigmented individuals of the ciliate, feeding on other organisms, would be found. The ciliate could be cultured free of the flagellate with relative ease.

If the association is permanent then it represents an unusual condition interpreted most readily as an obligate endosymbiosis in which the endosymbiont has undergone extreme degeneration. Division of the endosymbiont would have to be semisynchronous with its host. If a nucleus is absent from the endosymbiont, then, with the exception of the effects of the intraorganellar DNA, control of this process would lie with the ciliate. Ultimately it might be assumed that this could lead to a "phytociliate" possessing chloroplasts in the same manner as phytoflagellates. Most significantly, however, this new photosynthetic form would appear to be, by one of the principal criteria employed in current algal classification (pigment composition), a close relative of the cryptomonads! Clearly, if this type of organellar grafting from one branch of the evolutionary tree to another is possible, then it will be essential in future protist taxonomy to consider the host cell characteristics in parallel with the chloroplast characteristics.

In view of the potential significance of the case of *M. rubrum*, we regret being as yet unable to determine the true relation of the host cells to their pigmented bodies. This is due principally to the failure of all attempts to culture the species. We can simply sum up the evidence as it is known to us at present.

In his original description of *M. rubrum*, Lohmann (1908) included figures of colorless individuals, and assumed therefore that the presence of pigmented bodies was a temporary situation. He did not, however, discuss whether he considered the establishment of the chloroplastic state to occur at only one season, whether the bodies divided within the ciliate in synchrony with the latter's division, or whether, perhaps, there were permanently colorless individuals or strains. In fact, as we have mentioned earlier, the colorless specimens do not resemble the specimens we have seen. Instead they are very similar to descriptions of *M. pulex*, possessing a well-developed cytostome situated at the end of a conical oral pole. Consequently, either Lohmann wrongly included specimens of *M. pulex* in his original description of *M. rubrum*, or the pigmented cells are only a form of *M. pulex* and occur fairly frequently, the cells being morphologically modified in response to the photosynthetic condition. The latter view has been held by Bakker (1966, 1967a, b). This seems a reasonable possibility insofar as a temporary degeneration of the cytostomes, with rounding of the oral cone, might accompany the change to photosynthesis. However, the exceptional thinness of the pellicle, with a lack of alveoli, seems a major modification not likely to be easily accomplished on a short-term basis and its presence in the *pulex* forms would seem an improbable "preadaption." Only experimental evidence will confirm this hypothesis.

Most circumstantial evidence seems to favour the second hypothesis (permanent association). All the planktonic individuals that we and Fenchel (personal communication) have seen possessed morphologically similar chloroplasts and can be found at all times of the year. The ultrastructural features discernable in pictures of the Peru material sent to us by Dr S. Watson of the Woods Hole Oceanographic Institution indicate that the contained bodies are essentially identical to the British Columbia population. The host cells can remain alive for over a month in a light-equipped incubator. However, they die when subjected to dark periods in the incubator for 5 days, without noticeable digestion or egestion of the chloroplasts.

Although the pigment characteristics and the general shape of the chloroplast/mitochondrial complexes are highly suggestive of a cryptomonad, the thylakoid groupings within the chloroplast are unlike that of any species of cryptomonad as yet examined, all species of the latter group examined so far being found to invariably possess thylakoids grouped in closely appressed or unappressed pairs (Dodge 1969; Lucas 1970). Further, if Lohmann's (1908) observations of uniflagellate free individuals that he believed to be the endosymbiont and that he named *Erythromonas haltericola* are correct, this also does not conform with a cryptomonad identity. We doubt the common identity of the free cells and others of the same type observed by Lohmann in appendicularian "houses" with the cell derivatives present in *M. rubrum*. No other author appears to have seen *E. haltericola* since its discovery. Prior to the determination of the pigment characteristics the bodies were thought to be blue-green algae by Kahl (1930) and members of the Prasinophyceae by Parke (in Fonds and Eisma 1967).

The holotrich section of Kahl's (1930) monograph reveals many references to pigmented bodies described as either symbiotic cells or food bodies in the process of digestion. Holm-Hansen et al. (1970) have described a case of red water from Point Barrow, Alaska, caused by an unidentified species of holotrich (definitely not *Mesodinium*) that contained large quantities of chlorophyll *a*. However, the inclusions within it were too small to be recognisable as chloroplasts. Burkholder et al. (1967) reported on a bloom of ciliates, thought to be oligotrichs, from coastal waters in southern Puerto Rico. These ciliates contained brown pigmented bodies, fixed 289.7 mg C m^{-3} hr^{-1}, contained chlorophyll *a*, and had low assimilation numbers (between 1.0 and 1.4). We have made preliminary ultrastructural observations on several species of marine ciliates in which intact chloroplasts were present in the cytoplasm. They were not associated with any other structures that could be referred to as ingested flagellates. There were also signs of digestion of some of the chloroplasts. We suspect that these instances resemble the "temporary chloroplast maintenance" observed in opisthobranch molluscs mentioned earlier, and that this type of phenomenon may prove to be relatively common among marine ciliates.

There is little to discuss with regard to our general ultrastructural observations on *M. rubrum* as there is little basis for detailed comparisons with closely related species. The general arrangement of the organelles and, in particular, the type of mucocysts support the taxonomic assignment of the genus to the Didiniidae judging by the detailed studies that have been made on *Didinium nasutum* (Yagui and Shigenaka 1965; Dippell and Grimes 1966; Wessenburg and Antipa 1968). The lack of pellicular alveoli appears to be unique among ciliates (see Pitelka 1970), and the ciliary differentiations described are also extraordinary. Unlike *Didinium* the regions of the cortex free of cilia are also free of kinetosomes. The reduced cytostome and nemodesma in our specimens may not be typical for other species of *Mesodinium*, being probably associated with the presence of a photosynthetic nutritive system.

With regard to the infrageneric taxonomy there appear to be good grounds for the separate recognition of *M. acarus* at present in view of its distinctive, distally bifurcated cirri. It appears to use its cirri almost continuously to move with the cytostomal end foremost. From Dragesco's (1963) drawing of the chief ultrastructural features of *M. pupula*, together with its abundance in reducing marine sediments (Fenchel 1969), it appears reasonable to separate it from *M. rubrum*. The value of cytostomial tentacle characteristics may be weak if the tentacles are variably developed. This would not alter the situation regarding the separation of *M. acarus*, but would have bearing on the distinction of *M. pulex* from *M. rubrum* and *M. pupula*. If the photosynthetic condition of *M. rubrum* is facultative, and is accompanied by morphological changes such as proposed by Bakker (1966, 1967a, b) then *M. rubrum* is conspecific with *M. pulex*, the latter having priority. It can at least be asserted, as we have done earlier, that *M. rubrum* is conspecific with *C. meunieri* and that a designation to the genus *Cyclotrichium* would be inappropriate.

Little has been said yet of the role that *M. rubrum* plays within the ecosystem other than that it is a functional autotroph and thus can be considered a primary producer. Further we know of no noxious effects that have been reported in conjunction with blooms of this species, unlike dinoflagellate blooms in which a variety of unpleasant effects may occur. The only published information on feeding on *M. rubrum* is the brief note of Clemens (1935) in which a red pigmentation of the gill surfaces of oysters at the time of a bloom was taken as an indication of possible feeding on the ciliate. To this tentative record we can add that we have directly observed feeding on *M. rubrum* by a large as yet unidentified oligotrich ciliate, two species of cladocerans, *Podon leuckartii* Sars and *Evadne nordmanni* Loven, and one species of rotifer, *Synchaeta littoralis* Rousselet.

The exceptionally wide distribution of *M. rubrum* can be separated into two main types: one extremely neritic within bays and fjords; and one further offshore invariably associated with upwelled water. It is possible that the two situations optimise different parameters that give the species a competitive edge. Study of the Dutch Veerse Meer populations during the years 1964–67 (Vaas 1968) has led to the conclusion that salinity, temperature, and phosphate could be discounted as causes of blooms of the ciliate in those instances. Instead, a strong negative correlation between blooms and turbulence produced by westerly winds has been found. On the other hand, Fonds and Eisma (1967) produced reasonable evidence that linked blooms off the Dutch coast with phosphate-rich upwelled water resulting from easterly winds. One might certainly expect that, if vertical migration is a significant factor in the concentration of the organisms in the surface waters, vertical stability of the water would be a necessary prerequisite for red-water development. The question of nutrient supply for the blooms is inextricably bound up in the determination of the nature of the chlorophyll-containing bodies within the cells.

Clearly future research should be directed towards obtaining extended cultures of *M. rubrum* so that the association can be studied experimentally. Even without experimental manipulation it should be possible to observe whether the chloroplasts divide synchronously with the host. As many different, geographically separated populations as possible should be examined, particularly with the electron microscope, to determine the degrees of structural variation of the ciliate and its pigmented bodies. Also, much remains to be elucidated in the ultrastructure of the North Pacific population, especially with regard to the fibrillar structures.

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Satiation Time, Appetite, and Maximum Food Intake of Sockeye Salmon (*Oncorhynchus nerka*)

J. R. BRETT

Fisheries Research Board of Canada
Biological Station, Nanaimo, B.C.

BRETT, J. R. 1971. Satiation time, appetite, and maximum food intake of sockeye salmon (*Oncorhynchus nerka*). J. Fish. Res. Bd. Canada 28: 409–415.

Three aspects of feeding relations in sockeye salmon (*Oncorhynchus nerka*) were studied, using Abernathy pellets. When food was presented in excess at 15 C, mean satiation time was found to be 43 ± 8 min, independent of fish size. Voluntary food intake (appetite) increased with time, approaching a plateau after 25–30 hr deprivation. The greatest increase in appetite occurred between 7 and 11 hr of fasting. The relation between the capacity of the stomach (% body weight) and fish weight (grams) was exponential ($Y = 14.1–4.95 \log_{10}X$). Five-gram fish consumed 11% dry body weight at one feeding, and 17% on a daily basis.

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WITHIN the genetic potential of any species to grow, many abiotic and biotic factors limit maximum growth (Brown 1957; Ivlev 1961). The daily rate at which food can be consumed is a prime factor. This in turn is related to the capacity of the stomach and the rate of digestion.

In conjunction with experiments on gastric digestion in fingerling sockeye salmon, *Oncorhynchus nerka* (Brett and Higgs 1970), the question of maximum food intake and its relation to fish size arose. Before this could be studied with insight an assessment of the time required to reach satiation and the time sequence in the development of appetite was necessary. The following experiments were designed to help elucidate these problems. Each of the three interrelated aspects of feeding is described and discussed separately, followed by general conclusions.

Satiation Time

METHODS

The same culturing and holding techniques (45-gal fibreglass tanks) described in Brett et al. (1969) were used. Three groups of sockeye widely different in size (range 2–350 g) were fed an excess ration of Abernathy pellets (Fowler and Banks 1969) at exactly 9:00 AM each day for 3 weeks (see Table 1). Temperature was maintained at 15 ± 0.3 C. On the day of testing, weighed amounts of food were presented every 2 min for 1 hr. Uneaten particles were trapped in outlet cone-shaped screens, which were quickly removed and replaced every 2 min to allow for weighing the unused food. To

TABLE 1. Satiation times (min) for three sizes of sockeye fed on Abernathy pellets at 15 C. See text for basis of "corrected" times.

No. tests	Avg wet wt (*g*)	Pellet size[a] (*inch*)	Pellet moisture (%)	Satiation time 50%	75%	100%	Corrected time (100%)
1	1.93	3/32	33.6	9	21	54	50
1	25.9	3/32	33.6	4	14	37	33
3	236	1/8	27.4	6	21	48(43–56)[b]	44
			Mean	6.2	19.2	47.2	43.2
			SD	1.9	5.0	7.9	7.9

[a]Pellets are cylindrical in shape with diameters and lengths approximately the same. They are sold according to size in fractions of an inch.
[b]Range.

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accommodate an initial high rate of feeding, food was presented four times faster during the first 6 min than in the remainder of the feeding period (see slopes of lines in Fig. 1). The rate and amount of food supplied were set by preliminary observations on the quantity usually required to satisfy voluntary feeding on a once-daily regime. Fish were considered satiated when they would no longer accept any food, in the presence of excess, after a period of active feeding. The time from the start of feeding to voluntary cessation was defined as the "satiation time."

Results and Discussion

Since the flushing out of uneaten pellets varied with feeding rate, fish activity, and flow rate through the tank, the results are best examined as cumulative totals of food presented and food eaten (Fig. 1). Each value of the "food eaten" represents the difference between the amount of food presented and the amount washed out (excess). When these become equal, on the average, feeding has ceased and the fish are satiated. Estimated times to satiation and fractions thereof (50 and 75%) indicated initial rapid feeding followed by a steady diminution (Table 1). It was apparent that feeding rate responded to rate of presentation. Ideally fish should be provided with unlimited opportunity to feed. Experimentally they were presented, in a period of 1 hr, with food that totaled three to four times the amount consumed.

The time for 95% of a sample of pellets to pass through a standard tank *without* fish was approximately 4 min. At the end of an experiment *with* fish, clearance time (Fig. 1) was somewhat longer because pellets were seized, momentarily held, and rejected by the fish as satiation was approached. This results in a slight time delay (error) when calculating food consumed as the difference between input and output. The error is minimal at the start when most food is consumed, and maximal at satiation time. Hence only the final time (100% satiation in Table 1) was corrected, by 4 min, to provide a mean satiation time of 43 ± 8[1] min for all sizes. The longest time to reach satiation (50 min) occurred with the smallest fish. These consumed all that was supplied during the first 12 min, and may have been slightly influenced by a relatively insufficient initial rate of presentation.

Jack mackerel (*Trachurus japonicus*) and rainbow trout (*Salmo gairdneri*) feeding on mackerel meat and "compound feed" at 25 and 10 C, respectively, required 60 and 65 min to reach satiation (Ishiwata 1968). When expressed in the form of log F = $a + b$ log T, where F is the total food eaten and T the time, the data for these species approximate a linear relation, approaching a plateau at satiation. Assuming an equal and unlimited opportunity

[1]Standard deviation used throughout unless otherwise stated.

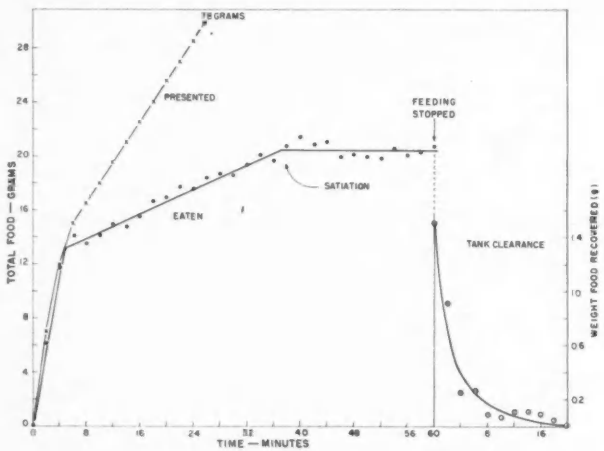

FIG. 1. Cumulative totals of food presented every 2 min and food eaten by 26-g sockeye at 15 C. Variability of points around mean rates of feeding results in part from variability of clearance time of uneaten pellets washed out of the tank during feeding. Final clearance of pellets from tank, after feeding is stopped, is indicated at right — note that the weight scale is expanded 10 times.

to feed, these results are in general agreement with those for sockeye salmon. However, two other species studied by Ishiwata (1968), the puffer (*Fugi vermicularis*) and the filefish (*Stephanolepis cirrhifer*), were satiated within 6 and 13 min, indicating a marked species difference.

Stretch receptors in the stomach wall constitute one of the mechanisms controlling the appetite of vertebrates (Lepkovsky 1948; Paintal 1954). Consequently the size of individual particles will have some effect on the point at which further distension is declined. In addition, animals tend to eat to satisfy their energy demand so that the caloric content of the food will also affect the size of daily ration (Mayer 1955; Rozin and Mayer 1961). By comparing the consumption of similar diets of small pelletized form, the relative effect of fish size could be studied within a fixed feeding regime, not complicated by differential pellet size or caloric content, or both.

It can be concluded that a 60-min feeding time would be quite adequate to satiate sockeye salmon, independent of size. This feeding time was used when conducting the following experiments but not during the introductory culturing period of 1-2 months in the laboratory.

Appetite

Methods

Voluntary food intake has been used as a measure of appetite in insects (Holling 1966), fish (Beukema 1968), and mammals (Miner 1955). It permits an objective assessment of hunger by recording the amount of food consumed in relation to the deprivation time. When periodically provided with excess food, repeated satiation occurs.

In the first tests on sockeye, using four tanks each with 25 fish (51 ± 5 g), food was presented at deprivation times corresponding to multiples of 2 hr up to 34 hr. The schedule was arranged so that no feeding occurred during the daily 8-hr dark period. From inspection there appeared to be a tendency for heavier feeding in the evening, interacting with deprivation time. The possibility that induced or circadian rhythms could be present required a change in procedure (cf. Pittendrigh and Bruce 1959; Hirata and Kobayashi 1956). The light intensity was altered to provide 0.6 cp during the dark period, approximately 1/50 that of the light period. For 3 weeks the fish were fed to near satiation once a day using a random choice for time of day. Feeding included the low-light "dark period" that was found to provide adequate visibility since the average amount consumed was not below that for the high-light period. The tanks were then programmed in pairs on 11-hr and 22-hr of deprivation, and maintained on this schedule for 12 consecutive feedings. In this way the time of feeding advanced 2 hr every day until a complete cycle around the 24-hr day had been represented.

Since the moisture content of fish and food varies, food consumed is expressed as a fraction: dry food/dry fish × 100, i.e., as "% dry body weight" (Fig. 2–4, 6). Drying was conducted in an oven at 105 C for 24 hr.

Results and Discussion

In the 11-hr series the sizes of two successive meals were inversely correlated ($r = -0.56$, $n = 47$, $P < 0.01$), independent of time of day (Fig. 2). A relatively large meal was usually followed by a relatively small meal, and vice-versa. The oscillations were most pronounced in the first 2 days and decreased to a smaller more consistent oscillation by the 3rd day, which is indicative of the time to adapt to the new feeding regime. No systematic order was apparent in the variability of food intake for the 22-hr series, in regard to either time of day or the influence of an above-average feeding ($r = +0.72$, $n = 23$).

At 15 C the sockeye stomach is virtually clear of food within 22.6 hr (Brett and Higgs 1970). Consequently on a 22-hr feeding cycle, appetite was little influenced by undigested food in the stomach. In the case of the 11-hr interval a residuum of about 10% of the contents remains. The size of a meal, therefore, affected the subsequent intake. This was particularly pronounced in the first 11-hr period after the relatively large intake characteristic of single daily feedings at 15 C (Fig. 2).

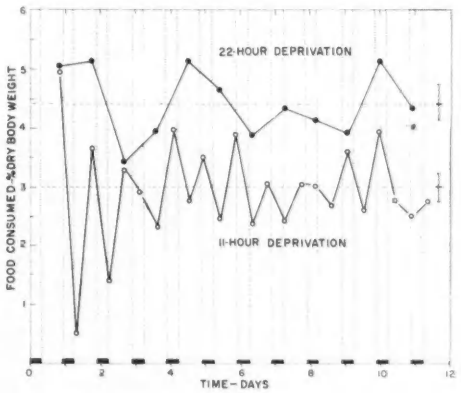

FIG. 2. Food consumed by 25 sockeye fingerlings after successive intervals of 11 or 22 hr of food deprivation. The mean±2 SE of the amounts consumed is shown by broken line and vertical bar. The dark phase of a 16-hr photoperiod was illuminated at 1/50 of the intensity of the light phase.

Appetite increased in a sigmoid pattern with time, approaching a plateau after 25–30 hr (Fig. 3). When compared with the progress of gastric digestion there is an obvious delay in the development of appetite during the first 6 hr. The greatest increase occurred between 7 and 11 hr deprivation when the stomach contents were respectively 75 and 90% digested.

Windell (1966) noted that the appetite of bluegill sunfish, *Lepomis macrochirus*, increased to a maximum after 4 days starvation and then decreased, accompanied by some degenerative changes in the pyloric caeca by the 10th day. A continued rise in appetite after 34 hr deprivation may also occur for sockeye. Emptying of the intestine contributes to increased appetite (Mayer 1955). However, it is apparent from the close relation with gastric digestion that the major controlling factor is the emptying of the stomach, which, as stated, occurred at about 23 hr for sockeye at 15 C.

Since the rate of change of appetite passed through an inflection point at about 11 hr of deprivation, this turned out to be a propitious choice for examining the effect of multiple daily feedings. It is the theoretical point at which the greatest degree of appetite has developed for the least deprivation time. The mean daily intake was 4.83% dry body weight/day[2] on a 22-hr schedule and 6.52% dry body weight/day on an 11-hr schedule. Because the amount consumed in a day was influenced by the time sequence in development of appetite, it can be seen that by controlling both meal size and feeding time a maximum daily intake could be achieved that would possibly double the voluntary food intake over that for a daily single presentation. This is indicated by the plot in Fig. 4, which shows that a restricted ration of 3% dry body weight fed at 11-hr intervals would enhance the daily intake. Further it shows the general possibility of optimizing daily food intake and presumably growth rate. Some loss in conversion efficiency might occur. Nonetheless the principle is clearly demonstrated.

Maximum Food Intake

The literature on the stomach contents of fish is replete with records of observed "mass" — by volume, wet weight, and, less frequently, dry weight. Relatively few studies have been made of the stomach capacity. Ivlev (1961) and LeBrasseur (1969) found widely different maximal rations depending on species, diet, and food or prey concentration. Thus, carp (*Carassius carassius*) feeding on bream roe consumed as much as 21% of their body weight, whereas roach (*Leuciscus rutilus*), on a diet of chironomid larvae, ate 15% in a 2-hr period; young chum salmon (*Oncorhynchus keta*) reached a maximum capacity of 5% when feeding

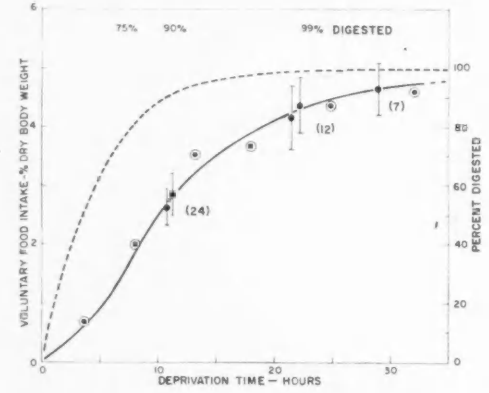

FIG. 3. The relation of food-deprivation time to appetite of sockeye fingerlings (51 ± 5 g). Number of replicates for each uncircled point indicated by numerals in parentheses (±2 SE); otherwise values are averages of three observations. Broken line represents the extent of digestion at 15 C (Brett and Higgs 1970).

[2]Note that this is expressed in terms of 24 hr, not 22 hr as in Fig. 4.

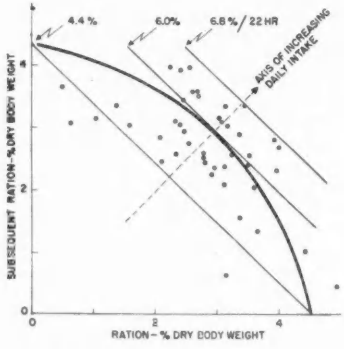

FIG. 4. Relation between size of a given meal and the subsequent intake when on an 11-hr feeding interval at 15 C. Elliptical curve was drawn by eye, passing through the means of x and y. Solid diagonal construction lines are the loci of feeding rates that sum to the selected 22-hr total intakes shown, e.g., every position along the first diagonal line represents the total of $x + y = 4.4\%$. On the average when a ration of 4.4% dry body weight is fed, little or no food is accepted 11 hr later. If a ration of 3% is fed, an additional 3% will be consumed 11 hr later.

on a small crustacean. Beukema (1968) drew up a table of daily food intake for seven species (weighing 1.3–4.6 g), which ranged from 5 to 13% body weight. Skipjack tuna, *Katsuwonus pelamis*, freely feeding on a fish diet, filled their stomachs to about one-third to one-half the maximum, total daily intake of 15% body weight (Magnuson 1969). From numerous records of adult Pacific salmon (500–3000 g) caught at sea, LeBrasseur (MS 1965) reported that the stomach contents of sockeye and pink salmon (*O. gorbuscha*) occasionally exceeded 5% of the fish's body weight; the average maximum contents were 2.2 and 2.5% for sockeye and pink salmon, respectively.

Apparently the daily intake of food is a variable quantity, which for a given species will depend on the mass ingested and the rate of processing (governing return of appetite). The relation is obviously influenced by fish size. In the extreme instance of a starving predaceous fish, the pattern of feeding may lead to degrees of stomach distention that considerably exceed that of the maximum capacity exhibited by the daily particulate feeder. The following experiments apply to regular daily feeding on small particles.

Methods

Three groups of 20 fish with mean wet weights of 4.4 ± 0.8 g, 44 ± 11 g, and 216 ± 75 g were cultured at 15 C on 1/32″, 3/32″, and 1/8″ Abernathy pellets. They were fed recorded amounts three times a day (8:30 AM, 4:30 PM, and 10:30 PM) for 20 min. On the final day they were fed once at midmorning for 1 hr. Fifteen minutes after the last feeding the fish were killed (100 ppm, M.S. 222), weighed to the nearest 0.01 g, and immediately deep-frozen. Subsequently all stomach contents were removed in the frozen state and weighed to the nearest 0.01 mg. One-half the sample was then used for volume determinations and the other half for dry weights.

Because of the fullness of the stomachs, some dying fish regurgitated food leaving some of the contents in the oesophagus. Most of these could be identified on dissection, necessitating the discard of 22% of the records.

An alternative method of inflating the stomachs of fish to the bursting point, as practiced by Kariya et al. (1968), appears to provide a useful physical dimension but is hard to conceive as reflecting the behavioural relation associated with voluntary food intake.

Results and Discussion

Moisture content of the pellets varied from 27.4% (1/8″ size) to 35% (1/32″ size). The fish averaged between 75 and 78% moisture.

Either from the drinking of water or the secretion of gastric fluid, or both, the stomach contents were over twice as moist as the pelleted food, resulting in a density of 1.08. Since the fish, with swimbladder punctured, had a similar density, there was no appreciable difference between determinations based on wet weights and those on volumes. Consequently, only the results for wet and dry weight measurements will be considered.

Maximum single intake

The amount of food in a "full" stomach varied from 3 to 13% among the small fish (3–6 g), and from 1 to 5% among the large fish (150–350 g) (Fig. 5). The variability can be attributed to three sources: (1) difference in stomach capacity (morphologically and physiologically); (2) reluctance of some fish to fill their stomachs at every feeding (behavioural); and (3) difficulty in handling the minute stomach contents of small fish (technical). The latter two sources introduce a distortion in the distribution of points by an error that tends to depress the mean values below the potential stomach capacity. A skewed frequency distribution results that was most pronounced for the small fish (Fig. 5B). The central tendency of the stomach-content data was therefore determined by finding the mode and mean ± 1 SD within each size group and plotting this in relation to the mean weight of the sample. Stomach capacity appeared to be an exponential function of size permitting a linear transformation over the range studied (Fig. 5C).

Maximum daily intake

Since the pellets were not the same moisture content as the fish, the amounts consumed were considered in terms of dry weights. The total daily intake decreased as a log–log function of dry body weight falling from an average of 16.9% (n = 5) for the 4-g fish to 4.3% for the 216-g fish (Fig. 6). When compared with the maximum single feedings[3] the smaller fish had an enhanced ability to consume more food than the larger fish (in terms of fraction of body weight), not only at a single meal but to a greater extent as multiple daily meals.

Despite the somewhat limited number of observations and the naturally large variability that accompanies feeding responses, the relation of size to the single and multiple daily intakes was quite systematically arranged according to the equations in Fig. 6. By extrapolation this results in a predicted ability of a 1-g sockeye (early lake-resident fry) to have a maximum single intake of

[3]Computed for one-half the values in Fig. 5, and not significantly different from the wet-weight relation.

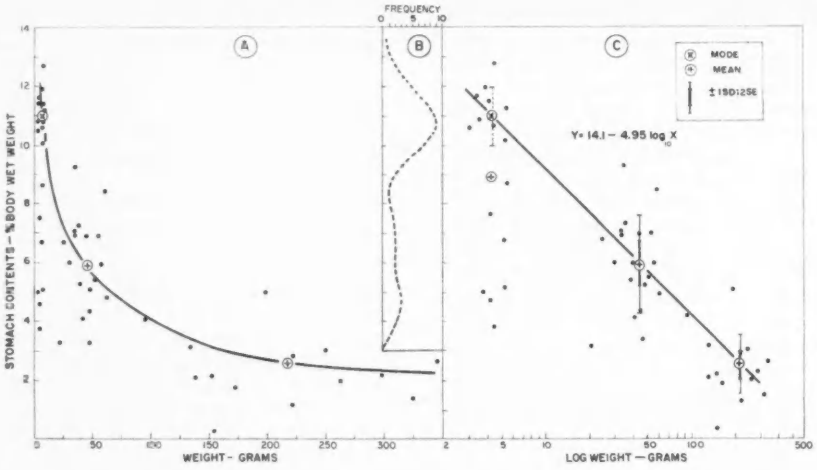

FIG. 5. Stomach capacity of sockeye salmon in relation to fish wet weight when on a daily satiation ration of pelleted food. The curve in A was drawn from the linear transformation in C. The central tendency of the latter was determined by using the mode for the small fish (mean weight 4.4 g) from the frequency plot in B. The mode and the mean coincided in the other two size groups, for which error estimates were plotted at the mean group weight. For the small fish the standard deviation was determined for the prime modal group only.

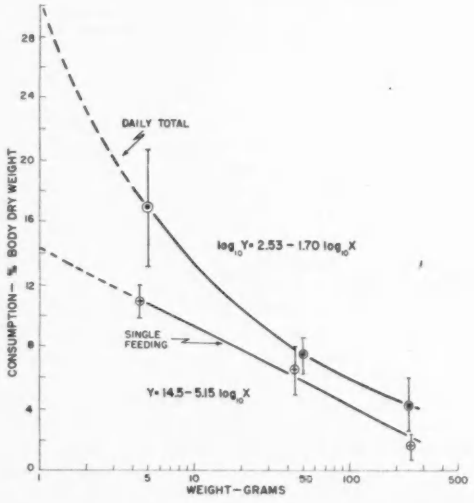

FIG. 6. Maximum food intake of sockeye salmon for single and triple daily feedings (daily total) in relation to fish wet weight. Mean consumptions (±1 SD) are shown with calculated extrapolations. Note that the slight change in the equation for "single feeding" results from the use of *dry* weight (cf. Fig. 5).

14.5% dry body weight and a daily intake of 30% at 15 C. Daily intakes of this order (particularly in nature) have only rarely been recorded in the literature, reflecting both the unusual circumstance and the possibility of error from extrapolation. Nevertheless, Davis and Warren (1968) found young chinook salmon, *Oncorhynchus tshawytscha* (0.6 g), would consume 20% body weight/day, and Krivobok (1953, cited in Winberg 1956) obtained daily rations as high as 54% dry weight in very young carp (0.015 g). Since growth rates of 30%/day and over are included in Winberg's (1956) review of food requirements of fish it is apparent that the figure of 30% for sockeye fry daily intake is not unreasonable.

Concluding Comments

The conclusion that size itself does not affect satiation time but does have a large influence on food intake must be tempered in the perspective that reduced capacity to grow may be acting to inhibit larger daily intakes among older larger fish. Thus the size-dependent rate of feeding and transforming energy could simply be a reflection of the size-dependent nature of growth rates.

It is generally apparent that food is processed into flesh much faster by a biomass of many small

fish than by an equal biomass of a few large ones, not only by an increased intake but also by some increase in the processing rate. In terms of cropping, assuming that the prey was equally available to any member within the size range, smaller members would be more effective harvesters.

In the promotion of growth both the size of ration and time of presentation can be favorably manipulated to maximize the daily intake by taking advantage of the earliest point at which appetite is approaching a peak. This corresponded to a state of 90% evacuation of the stomachs in young sockeye.

Acknowledgments

Collection of much of the basic data and preliminary analysis was performed by Mr C. Zala, a student in the Department of Biochemistry, University of Victoria, Victoria, B.C. His perceptive interest and ardent capacity for work provided considerable stimulus to the conduct of the research.

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Reproductive Patterns of Pacific Ocean Perch (*Sebastodes alutus*) off Washington and British Columbia and their Relation to Bathymetric Distribution and Seasonal Abundance

DONALD R. GUNDERSON

Washington State Department of Fisheries
Fisheries Center, University of Washington, Seattle, Wash. 98105, USA

GUNDERSON, D. R. 1971. Reproductive patterns of Pacific ocean perch (*Sebastodes alutus*) off Washington and British Columbia and their relation to bathymetric distribution and seasonal abundance. J. Fish. Res. Bd. Canada 28: 417–425.

Females of the ovoviviparous species *Sebastodes alutus* release their larvae in March in the Washington–British Columbia area. Male maturity data from Queen Charlotte Sound indicated that males probably inseminate females in September or October. Examination of the annual bathymetric cycle in three major commercial trawling areas showed that mating and insemination occur while Pacific ocean perch are moving to deep water from the shallow-water regions inhabited in summer and that females release their larvae while in the deepest part of their bathymetric range.

Total 1955 through 1965 catch by month, and 11-year mean monthly catch rates were used to indicate relative seasonal abundance on major fishing grounds. Where deepwater regions were trawled, these indices showed a spring maximum associated with the March spawning period. In all areas studied there was a midsummer slump in catch and catch rate, and an October maximum occurring during the suggested mating period.

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PACIFIC ocean perch (*Sebastodes alutus*) are found from La Jolla, California, to the Bering Sea. *Sebastodes alutus* is an ovoviviparous species and has three major processes in its reproductive cycle. These are: mating (when spermatozoa are transferred from males to females), fertilization (when the ova are actually fertilized), and spawning (when the larvae are released).

Migrations of Pacific ocean perch have been studied in the Bering Sea (Paraketsov 1963), the Gulf of Alaska (Lyubimova 1963, 1964, 1965), and off the Washington coast (Alverson 1960). In all of these areas migration seemed to be related to the reproductive process, with the fish moving into deepwater spawning areas to release their embryos, then returning to shallow water while the gonads ripen. In the Bering Sea and Gulf of Alaska, mating occurs just before the migration to deep water.

The purpose of this paper is to document the migration pattern of Pacific ocean perch off the Washington–Vancouver Island area more thoroughly than it has previously been done and to correlate this pattern with the reproductive cycle of *S. alutus* in this region.

Methods

In this study catch statistics of the Washington trawl fleet were the basic data source used in establishing the migration pattern of Pacific ocean perch, whereas maturity observations were used to study their reproductive pattern.

CATCH STATISTICS

The Washington trawl fleet was the major exploiter of Pacific ocean perch stocks off Washington and Vancouver Island prior to 1966 (Table 1). In 1966 USSR and Japanese trawl fleets first appeared in these areas in significant numbers, relegating American fleets to a secondary role.

The Washington trawl catch statistics used in this study are of two types — interview and final. Interview statistics originate from data supplied by trawler captains on depth and total hours fished in a given area as well as their estimates of the total poundage caught for each species. Actual pounds landed are obtained by species from buyer's receiving tickets but data on effort expended in making these catches and their geographic origin are available only through interviews.

Final catch statistics are obtained by correcting fishermen's estimates of poundage caught and extrapolating interviewed effort data to the catch of the uninterviewed portion of the fleet. Alverson (1956) gave a more thorough description of the methods used in obtaining these statistics.

Final statistics were used to investigate seasonal variations in catch and catch rate. Such data were not available by depth, and interview data that had been corrected for fishermen's estimating errors were consequently used to study bathymetric catch patterns. Although factors such as fishermen's behavioral patterns, weather, market conditions, and relative abundance

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of more desirable species can result in a failure of landings and catch rates to reflect changes in abundance, it has been concluded by Alverson (1960) that the trawl fishery probably reflects general seasonal trends in the availability of several species. Long-term changes in efficiency and intensity of fishing are of minor importance when examining relative seasonal abundance or bathymetric distributions.

Although use of catch statistics as indicators of abundance would be unwarranted for lightly exploited species, it is justified for Pacific ocean perch because a firm market exists for them and they are actively sought during all times of the year. Demand for Pacific ocean perch has grown steadily since 1955 and during the period from 1960 to 1966 this species comprised 27% of the total Washington trawl foodfish landings.

Three geographic regions, each representing a major Pacific ocean perch ground, were chosen for analysis of catch statistics. Washington statistical areas 2 and 3 (Fig. 1) were grouped to form the Queen Charlotte Sound area, statistical areas 6, 7, and 8 were grouped to form the Estevan area, and statistical area 13 made up the Cape Flattery Spit area. Landings from these three geographic areas accounted for 80% of the total Washington Pacific ocean perch catch for the years 1955 through 1965.

Maturity Studies

Male and female Pacific ocean perch from fresh commercial catches were examined for maturity during 1968 and 1969. Maturity stage was classified by the criteria in Table 2 after Westrheim et al. (MS 1968). All data on male maturity were obtained during 1969 when the occurrence of male maturity stage 3 (large white) was first recognized and recorded. A similar stage of male maturity was described for *S. paucispinis* by Moser (1967a), who described an enlarging and whitening of the testes during the final states of spermatogenesis. During this stage he found large cysts of spermatozoa scattered throughout the testes.

The date of sampling the landings lagged from 2 to 10 days behind the date the fish were actually caught. The majority of the commercial catches originated in Queen Charlotte Sound (Fig. 1). Commercial catches from Cape Flattery Spit were available for only a short time in the spring. To supplement data for this area, maturity observations made aboard the University of Washington research vessel *Commando* and the Bureau of Commercial Fisheries research vessel *Miller Freeman* were incorporated into the study.

Results

Bathymetric Cycles

The 1961 through 1965 bathymetric cycles for *S. alutus*, as indicated by weighted mean monthly depth of catch, are shown for three specific areas off Washington and Vancouver Island in Fig. 2. Little importance should be attached to the erratic 1961 data for Queen Charlotte Sound as the fishery was then of an exploratory nature.

Before Fig. 2 can be properly interpreted, one must realize that it is frequently the availability of trawlable ground rather than the availability of fish that determines maximum depth of fishing. In Queen Charlotte Sound an abrupt dropoff exists past 150 fath, a fact that is reflected by the historically small fraction of the *S. alutus* catch there being made at depths greater than 149 fath (Fig. 3). In contrast, Fig. 3 shows that at Estevan and Cape Flattery Spit grounds significant catches are made at depths exceeding 150 fath. At Estevan a much greater proportion of the annual catch comes from 169 fath and deeper, than from the corresponding depths at Cape Flattery Spit, probably due to the rough nature of the bottom in the latter area.

Considering the effect that trawlability of grounds has on maximum depths fished, the 5-year mean bathymetric patterns of *S. alutus* catches from the three areas studied are quite similar, although a certain amount of variation is present from year to year (Fig. 2). The fish are at the shallowest point in their bathymetric cycle during June through August, during which mean monthly depth of

Table 1. Combined Pacific ocean perch catch of Washington, Oregon, and Canadian trawl fleets vs. Washington catch for 1956–65, taken from Washington and Vancouver Island coasts and Queen Charlotte Sound.[a]

	1956	1957	1958	1959	1960	1961	1962	1963	1964	1965	Total
Washington, Oregon, Canadian catch (*000's of lb.*)	5953	6020	4669	6972	8225	10140	15416	19999	15588	20193	113175
Washington trawl fleet catch (*000's of lb.*)	4797	4047	2698	5834	5999	7867	11432	15560	11274	14276	83784
% of combined catch	81	67	58	84	73	78	74	78	72	71	74

[a]Data from Pacific Marine Fisheries Commission bottomfish series, areas 2D–5B.

catch varies from 90 to 120 fath. About September, the weighted mean depth of catch starts to increase as the fish move into deep water. During December through April, *S. alutus* are at the deepest part of their bathymetric range and starting about May the mean monthly depths of catch start to decrease as the fish return to shallow water. This is similar to the bathymetric cycle indicated for the area off the Washington coast by 1955 through 1957 mean depth of catch data (Alverson 1960). The time of return from deep to shallow water seems to be the only aspect of the bathymetric cycle showing obvious variation between areas, consistently occurring as early as April in Queen Charlotte Sound and as late as June in the Estevan area.

Cycle of Abundance

Seasonal variations in the abundance of *S. alutus* stocks on the fishing grounds should cause corresponding variations in the 1955 through 1965 catch by month and mean monthly catch per hour statistics, assuming the trawl fleet is effective in locating and fishing all concentrations of fish on trawlable grounds. The seasonal cycle of abundance indicated by catch and catch per hour data is shown for Queen Charlotte Sound, Estevan, and Cape Flattery Spit in Fig. 4.

Although the maturation and bathymetric cycles are similar for these areas, the cycle of abundance is markedly different in Queen Charlotte Sound

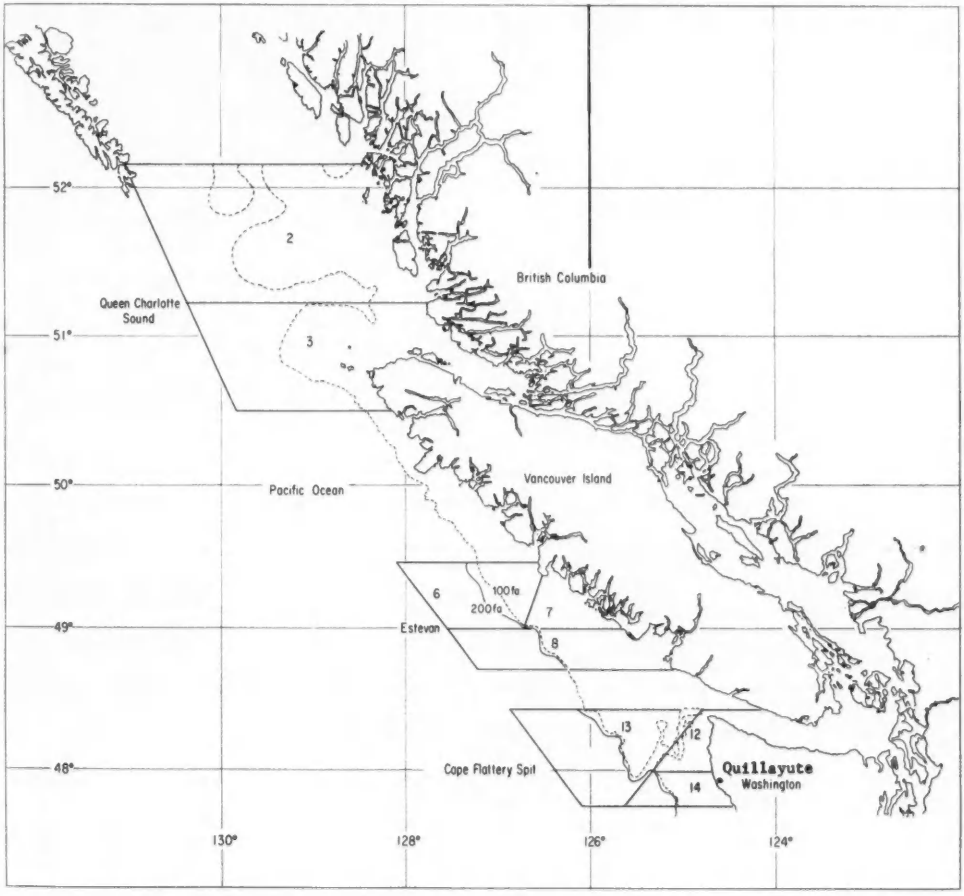

FIG. 1. Washington and Vancouver Island coasts. Each of the three major areas studied, as well as its component statistical areas, are outlined.

than in Estevan or Cape Flattery Spit. This difference is a reflection of the depths fished, since in both of the latter areas a significant deepwater fishery takes place in the spring, whereas for the most part the grounds fished in Queen Charlotte Sound are no deeper than 150 fathoms (Fig. 3).

TABLE 2. Description of maturity stages of *S. alutus* gonads.

Maturity stage	% in maturity stage	Description
		Males
Resting	8	Ribbonlike, triangular in cross section, brown or white
Large white	3	Large and swollen, somewhat rounded in cross section, glistening white
		Females
Large yellow	3	Ovary firm, ova yellowish and opaque
Yolk cleared	4	Ovary not firm, ova yellowed and translucent
Eyed embryos or larvae	5	Ovary not firm, ova translucent yellow with black dots or opaque grey with black dots
Spent	6	Ovary large and flaccid with a red, purple, or dark grey color
Resting	7	Ovary firm, grey or pink colored, some with black blotches

It can be seen in Fig. 4 that for Estevan and Cape Flattery Spit, where regions 180 fath and deeper are fished, the spring peak in abundance occurs in March. In Queen Charlotte Sound the spring maximum occurs in May, as fish are returning from deep to shallow water.

After the spring peak in abundance, landings and catch per unit of effort values show marked

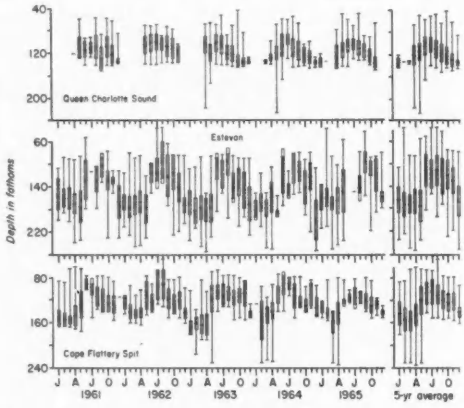

FIG. 2. Weighted mean depth of catch by month for Pacific ocean perch from Queen Charlotte Sound, Estevan, and Cape Flattery Spit, 1961–65. (Data from Washington Statistical area 12 were added to that for area 13 for the first 5 months of 1961. The two statistical areas were not differentiated during that time.) Horizontal lines on bars, means; vertical bars, standard deviations; vertical lines, ranges.

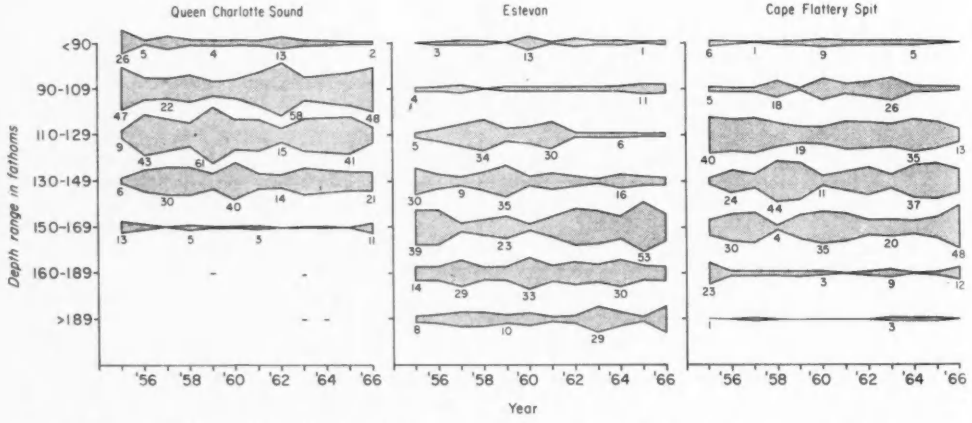

FIG. 3. Percentage of annual *S. alutus* catch by depth for Queen Charlotte Sound, Estevan, and Cape Flattery Spit–Quillayute grounds, 1955–66. (Washington statistical areas 12, 13, and 14 comprise the Cape Flattery Spit–Quillayute grounds.) Expressed in volume percentage.

declines in all areas. These declines seem to be centered around the June, July, and August period, when Pacific ocean perch are at the shallowest point in their bathymetric cycle (Fig. 2). The exact timing of this slump is somewhat variable, depending on the area involved and the abundance index given the most credence.

In all areas there was a fall peak in abundance occurring about October, as indicated by trends in the catch, although catch per hour data showed this peak to occur from September to January. Monthly trends in catch per hour showed much greater variation between areas than did trends in the catch, and less importance should probably be attached to them. Catch per hour data from Cape Flattery Spit did not seem to reflect the strong fall peak shown by trends in the catch and observed on other fishing grounds.

Lestev (1961) and Paraketsov (1963) did not mention any fall peak in research catches made in the Bering Sea, and reported that their largest catches were made on spawning schools of *S. alutus* in the winter and spring (March–May).

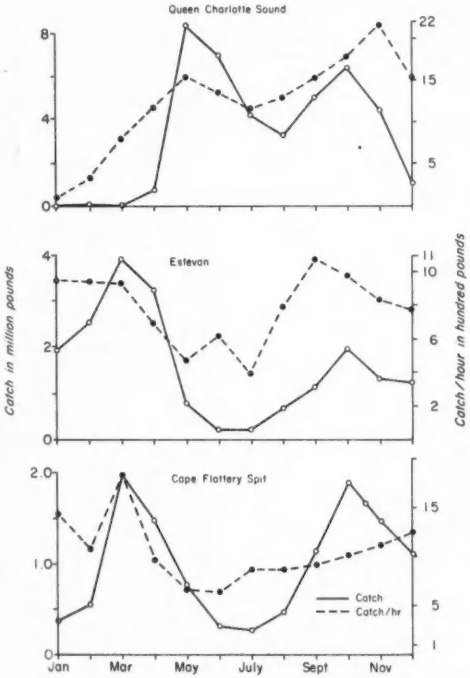

FIG. 4. Catch and catch per hour of Pacific ocean perch from Queen Charlotte Sound, Estevan, and Cape Flattery Spit, 1955–65.

Lyubimova (1963, 1964, and 1965) described a cycle of abundance that differs from either the Bering Sea or the Washington–Vancouver Island cycle. In the Gulf, highest research vessel catch rates were made when Pacific ocean perch moved through a given area on their seemingly extensive migrations, and spawning concentrations yielding large catch rates were never found.

MATURITY

The data in Table 3 indicate that for Queen Charlotte Sound, peak spawning occurs in March. At this time, the Pacific ocean perch fishery in this area is almost negligible (Fig. 4) and few commercial landings are available for sampling. The small amount of data that were available for February indicates that few females had released embryos before then. This agrees with Westrheim et al. (MS 1968), who examined 43 mature females from a February commercial catch made in Queen Charlotte Sound and found that 5% were in stage 3, 83% in stage 4, and 10% in stage 5, whereas only 2% were in the spent or resting stage. In the present study no data were available for March, but Table 3 shows that spawning was essentially completed by April since 98% of the mature females examined then were either spent or resting.

In the Cape Flattery Spit area, peak spawning again seemed to occur in March with 98% of the mature females examined in April being in either the spent or resting stage, whereas only 2% had been this advanced in February. The data for this area agree with those of Westrheim et al. (MS 1968), who found a March spawning time encompassing 30 days for *S. alutus* off southwest Vancouver Island. On the basis of limited data, Westrheim (MS 1958) had previously postulated a January or February spawning time for the Washington and Oregon areas.

Peak percentage of Queen Charlotte Sound males with large white testes occurred in August (Table 3). The percent occurrence of the large white maturity stage started to decline during September, corresponding to the decline in mean male gonadal weight reported by Moser (1967a) for *S. paucispinis*. Moser (1967b) showed that this decline resulted from loss of sperm due to mating and that the timing of the decline was coincident with the time that spermatozoa were first discovered during examinations of female reproductive tracts. Applying Moser's findings to *S. alutus*, the probable time of mating is September or October in Queen Charlotte Sound. This is substantiated to some degree by the parity of the sex ratios prevailing during the September–November period (Table 4)

and the fact that the catch of Pacific ocean perch in Queen Charlotte Sound shows a peak during this period (Fig. 4). Both of these phenomena are what would be expected if mating takes place on the bottom in trawlable areas, and the fish are congregating in these areas to mate.

Clearing of the yolk has been studied in many rockfish (DeLacy et al. 1964; Hitz and DeLacy 1965; Moser 1967a) and seems to be most commonly associated with maturing of the ova, ovulation, and early development of embryos. If *S. alutus* mate in September and October, and clearing of

TABLE 3. Percent maturity composition by month of landing for mature fish collected from two areas, 1968 and 1969.

	Queen Charlotte Sound (*87–165 fath*)										
	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
					Males						
Total examined			95	115	63	90	93	46	206	99	
% in each maturity stage 8			96	100	59	14	3	28	65	100	
3			4		41	86	97	72	35		
Total			100	100	100	100	100	100	100	100	
					Females						
Total examined	7		38	126	125	77	309	117	425	274	
% in each maturity stage 3	71				2	39	71	97	100	99	
4			3						[a]	1	
5											
6	14		11	26	4	3	1				
7	14		87	74	94	58	28	3			
Total	99		101	100	100	100	100	100	100	100	

	Cape Flattery Spit and Quillayute (*210–240 fath*)										
	Jan.[b]	Feb.[b]	Mar.[b]	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
					Males							
Total examined		124	153	129			120					
% in each maturity stage 8		100	100	100			22					
3							78					
Total		100	100	100			100					
					Females							
Total examined	54	89	178	363			129					
% in each maturity stage 3	30		1	1			78					
4	69	8	1									
5	2	90	65	1								
6		1	17	14								
7		1	16	84			22					
Total	101	100	100	100			100					

[a]Trace — less than 1%.
[b]All fish examined during this month came from research vessels.

TABLE 4. Percent males, by month, encountered in individual samples of commercial catches from Queen Charlotte Sound, 1966–69.

Month									Median % males	Mean % males
January										
Depth (*fath*)	142–150									
No. sampled	257									(257)
% males	42								42	42
February										
Depth	156–160	155	140–165							
No. sampled	150	309	150							(609)
% males	87	77	87						87	82
April										
Depth	140–150	100								
No. sampled	285	319								(604)
% males	93	66							80[a]	79
May										
Depth	115–125	104–130	95–120	120	135–140	100–105	137–150	123–125		
No. sampled	248	219	221	271	348	381	310	329		(2327)
% males	73	67	47	73	68	61	60	84	68	67
June										
Depth	88–133	98–100	90–120	100–105						
No. sampled	321	264	305	234						(1124)
% males	52	33	43	48					44[a]	43
July										
Depth	116	100–110	80–130	95–112	120–127					
No. sampled	297	319	248	231	157					(1252)
% males	42	46	27	38	48				42	40
August										
Depths	100–140	102–112	87–98	100–130	109	100–130				
No. sampled	298	285	252	333	306	226				(1700)
% males	47	40	40	36	46	48			43[a]	43
September										
Depth	120–140	112–125	103–132	115–120	110–120	110–115	112–145	70–105		
No. sampled	378	207	292	301	212	325	309	316		
% males	66	65	61	47	33	37	45	33		
Depth	130–145	82–147	130–140							
No. sampled	230	222	305							(3097)
% males	25	52	55						47	48
October										
Depth	153	133–135	160	135–160	130–150	130–140	140–160	118–140	110–140	
No. sampled	210	384	301	444	339	331	286	313	292	
% males	49	48	61	43	52	61	41	47	48	
Depth	125–130	118–135	115–135	125–132	105–113	125–135	120–140	135–165		
No. sampled	209	218	361	334	248	311	279	328		(5188)
% males	27	37	40	48	53	48	53	52	48	48
November										
Depth	112–130	145–158	138–150	152	125–140	150	120–159			
No. sampled	337	295	338	267	339	498	229			
% males	47	29	51	53	76	48	41			
Depth	150	117–150	140–158							
No. sampled	338	360	334							(3335)
% males	79	30	46						48[a]	50
December										
Depth	116–130	125–150	133–155							
No. sampled	306	301	265							(872)
% males	53	57	38						53	50

[a]Obtained by computing a number halfway between the two middle values.

yolk is associated with the actual fertilization of the eggs, between 1 and 4 months elapse between insemination and fertilization. This time lapse is probably less than 1 month for *S. paucispinis* (Moser 1967a) but has been estimated at up to 6 months for *Sebastes marinus* (Sorokin 1961), the redfish.

Sex Ratios

Some interesting changes in sex ratios of the commercial catch occurred during the study and seemed to be related to bathymetric migrations and spawning behavior. Although sex ratios from Queen Charlotte Sound sometimes vary widely between samples, Table 4 indicates the mean monthly proportion of males in the catches is lowest during the June–August period and that beginning in September, there is a gradual increase in this proportion. By February, the mean proportion of males was markedly higher than it had been during June through August, indicating that a significant fraction of the males do not accompany females to the spawning areas. Westrheim (MS 1958) presented data similar to those in Table 4, and although they probably originated from a wider range of areas and depths, they reflected the same trends. These findings agree with Lyubimova (1963, 1965) who observed even sex ratios in the Gulf of Alaska during the summer, but noticed that many of the males did not accompany females on their spawning migration. Paraketsov (1963) reported that males began to separate from females during the spawning season in the Bering Sea.

Discussion

In general, for those areas where a wide range of depths is exploited, the catch statistics reflect a March peak in abundance corresponding to the spawning period and the time when Pacific ocean perch are in the deepest part of their bathymetric range. For all areas there is a June–August slump in catch and catch per hour when most of the fish caught come from waters 90–120 fath deep, and a fall peak in abundance occurring from September to November. The fall peak occurs during or immediately after the suggested time of mating at a time when *S. alutus* are moving from shallow to deep water.

Changes in abundance on the fishing grounds probably resulted from a redistribution of *S. alutus* within the study area, rather than from the migration of a large stock of fish into and out of these areas. The data in Fig. 4 substantiate this somewhat, since it is unlikely that Pacific ocean perch make a simultaneous midsummer migration out of all three areas studied, go to areas where they cannot be detected by the fishery, and then return to the study area before the fall migration into deep water. Instead, it is probable that during June, July, and August the fish remain in the three areas studied, but become less available to the fishery either because they spend more time in midwater and untrawlable areas during this period or because they are more widely dispersed than at other times of the year.

If the assumption that changes in abundance on the fishing grounds result from a redistribution of *S. alutus* in the study areas is acceptable, then the changes previously discussed as changes in abundance could also be classified as changes in availability.

It is not necessary to hypothesize migrations of more than 10 miles to explain the bathymetric cycle reported in this paper for *S. alutus* (Fig. 1). There is no reason to discount the possibility that much more extensive migrations occur, however, and fish spawning at Cape Flattery Spit, for example, could spend the summer in areas several hundred miles away.

Acknowledgments

The author thanks Dr J. Reeves for his help in preparing data on bathymetric trends and H. H. Shippen (Bureau of Commercial Fisheries) for making certain maturity observations possible. C. H. Hitz and S. J. Westrheim gave valuable assistance in preparing the manuscript. This study was carried out with funds originating from P.L. 88–309.

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Comparison of the Pectoral and Pelvic Skeletons and of some other Bones and their Phylogenetic Implications in the Aulorhynchidae and Gasterosteidae (Pisces)

JOSEPH S. NELSON

Department of Zoology
The University of Alberta, Edmonton, Alta.

NELSON, J. S. 1971. Comparison of the pectoral and pelvic skeletons and of some other bones and their phylogenetic implications in the Aulorhynchidae and Gasterosteidae (Pisces). J. Fish. Res. Bd. Canada 28: 427–442.

Study of the pectoral and pelvic skeletons and some other bones in recognized species of the seven genera comprising the related families Aulorhynchidae (tubesnouts) and Gasterosteidae (sticklebacks) showed that the two families differ in several respects, with no one living species appearing to be intermediate. The genera *Aulorhynchus*, *Aulichthys*, *Spinachia*, *Apeltes*, and *Gasterosteus* each have distinctive characteristics not found in any other genus of the two families. *Gasterosteus wheatlandi* is the only species of the two families that lacks both the posttemporal and the supracleithrum. The interrelations of the genera form a mosaic pattern and there is no acceptable basis in the characters examined for postulating a phylogeny within the Gasterosteidae or for selecting a living species as being a primitive form or the most closely allied to the Aulorhynchidae.

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THE two fish families Aulorhynchidae (tubesnouts) and Gasterosteidae (sticklebacks) are considered to be closely related by virtually all ichthyologists (e.g., Gill 1863; Jordan and Gilbert 1883; Regan 1913; Berg 1940; Greenwood et al. 1966). Some workers even suggest that they might be combined (Gill 1885; Starks 1903; Limbaugh 1962; Reisman and Cade 1967), though none has formally proposed it. The interrelations of the five recognized genera of sticklebacks have drawn much speculation. Phylogenetic schemes put forth in this century suggest that either *Spinachia spinachia* or *Apeltes quadracus* is closest to the ancestral type and that these two species are relatively closely related (Bertin 1925; Leiner 1934; Hall MS 1956). Various workers have also regarded *S. spinachia* as being intermediate between the Gasterosteidae and Aulorhynchidae (e.g., Gill 1885).

The purpose of this paper is to present evidence from a reexamination of the pectoral and pelvic skeletons and of some other bones, which suggests that the Aulorhynchidae and Gasterosteidae form two separate evolutionary lines with no gradation between the two in any one living species, and to present some diagnostic features of the member taxa. Evidence will be presented that suggests that the interrelations within the Gasterosteidae are extremely complex and that there appears to be no basis in the characters examined for postulating any living species as representing the ancestral type or for holding that *S. spinachia* and *A. quadracus* are relatively close.

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Materials and Methods

The following material, received from the sources as noted, was examined (UAMZ = The University of Alberta Museum of Zoology). Uncatalogued material is in my care in the Department of Zoology. The stained and cleared material was prepared by the enzyme technique of Taylor (1967). Drawings were made with the aid of a camera lucida.

AULICHTHYS JAPONICUS GILL[1]

Kumanoto-Ken, Japan, M. Nakamura (one adult and eight young stained and cleared, seven unstained young).

AULORHYNCHUS FLAVIDUS GILL

Graham Island, B.C., UAMZ 1694 (three stained and cleared adults).

Burrard Inlet, B.C., UAMZ 915 (four stained and cleared young.)

Saturna Island, B.C., N. J. Wilimovsky (six stained and cleared young).

[1]The author of this species is generally given as Brevoort or as (Brevoort) Gill but Brevoort, as far as I can determine, never published his description and the first valid description of the genus and of the species is by Gill (1863).

Friday Harbor, Washington, N. J. Wilimovsky (four stained and cleared young).

Puget Sound, Washington, D. W. Hagen (15 stained adults).

SPINACHIA SPINACHIA (LINNAEUS)[2]

Norway, D. W. Hagen (stained series of young).

Isle of Man, Great Britain, A. B. Bower and R. H. Moore (three young and three adults stained and cleared, 10 unstained adults, series of unstained young).

APELTES QUADRACUS (MITCHILL)

Big Spring Run River, Pennsylvania (six stained and cleared adults, series of unstained adults).

Great Pond, Falmouth, Massachusetts, H. M. Reisman (eight stained and cleared adults).

Great South Bay, Long Island, New York, P. T. Briggs and R. H. Schaefer (series of unstained young and adults).

Solomens, Maryland, F. J. Schwartz (10 stained and cleared adults).

GASTEROSTEUS ACULEATUS COMPLEX[3]

Nimpkish Lake, B.C., (six stained and cleared adults).

Paxton Lake, B.C., J. D. McPhail (six stained and cleared benthic adults).

Reid State Park, Maine, H. M. Reisman (four stained and cleared adults).

Trynka River, Poland, T. Penczak (10 stained and cleared adults).

Ogaki, Gifu, Japan, K. Igarashi (four stained and cleared adults).

Takahama, Fukui, Japan, K. Igarashi (two stained and cleared adults).

Additional unstained material from Newfoundland (E. T. Garside and R. E. E. Moodie), Washington (D. W. Hagen and E. W. Wadley), Roumania (P. Banarescu), Czechoslovakia (O. Oliva), USSR (G. Nikolsky, A. N. Svetovidov, and A. A. Svetovidova), and Japan (M. Nakamura).

GASTEROSTEUS WHEATLANDI PUTNAM

Reid State Park, Maine, H. M. Reisman (27 stained and cleared adults. unstained series).

PUNGITIUS[4] PUNGITIUS COMPLEX[5]

Pine Lake, Alta., E. Kuyt (20 stained and cleared adults).

Round Lake, Sask., D. W. Hagen (10 stained and cleared adults).

Lake Superior, Wisconsin, T. D. Beard (10 stained and cleared adults).

Frisian Island Sylt and Hunnau Brook, Germany, J. Münzing (stained adults).

Riga Bay, Baltic Sea, A. A. Svetovidova and G. Nikolsky (three stained and cleared adults).

Sakhalin Island, USSR, A. A. Svetovidova and G. Nikolsky (three stained and cleared adults).

Takefu, Fukui, Japan, K. Igarashi (six stained and cleared adults, *P. pungitius sinensis* (Guichenot)).

Toro-Lake, Japan, M. Nakamura (five unstained adults, *P. pungitius sinensis*).

PUNGITIUS PLATYGASTER (KESSLER)

Danube Delta, Roumania, P. Banarescu (five stained and cleared adults).

Azov Sea, USSR, A. A. Svetovidova, A. N. Svetovidov, and G. Nikolsky (seven stained and cleared adults).

CULAEA INCONSTANS (KIRTLAND)

Much stained and cleared material from populations mentioned in Nelson (1969).

Terminology of Structures in the Pectoral and Pelvic Skeleton Region

Though pectoral and pelvic skeletons have been frequently used in taxonomic studies of the gastero-

[2]Fleming (1828 p. 219) erected the new genus *Spinachia* for Linnaeus' *Gasterosteus spinachia* and gave it a new specific name, *vulgaris*. The species since then has commonly been referred to as *S. vulgaris* Fleming but I can find no justification for this. The specific name *spinachia* has been used by a few authors after both the works of Linnaeus and Fleming.

[3]The taxonomic problems in this highly differentiated group make it desirable, for the present, to retain all units in one category while at the same time recognizing the biological diversity. Early workers recognized numerous species (Cuvier and Valenciennes 1829; Sauvage 1874 — synonymy in Jordan and Evermann 1896; Jordan et al. 1930). Subsequent to this gross splitting, all *Gasterosteus* forms were lumped into one variable species, *G. aculeatus* Linnaeus (Regan 1909; Bertin 1925). Recent taxonomic studies and experimental work on the morphology of this group (Heuts 1947a, b; Lindsey 1962a; Münzing 1959, 1963; Penczak 1966; Miller and Hubbs 1969) continue to reveal much variation, and studies on understanding the biological significance of much of the diversity (in part anticipated by Hubbs 1929) suggest the presence of many "biological" species within the group (Hagen 1967; McPhail 1969; Hagen and McPhail 1970; Moodie MS 1970).

[4]The generic name *Pygosteus*, first used by Gill (1862) from a manuscript of Brevoort, is still often used in the European literature. Lindsey (1962b) adequately notes the priority of Coste's (1846) *Pungitius*.

[5]The taxonomic problems here are similar to those in the *G. aculeatus* complex. As in the latter, freshwater and anadromous populations exist, with much morphological variation within each life-history type. Asiatic workers recognize more than one species in *Pungitius* and recent biochemical studies (Muramoto et al. 1969) tend to support this view.

steoids, little agreement in the terminology exists. The terminology for all bones mentioned in this study follows Norden's (1961) study of salmonids for comparable structures, except where noted. The following synonymy includes works only on tubesnouts and sticklebacks. The structures of the two girdles are shown in Fig. 1.

A: Actinosts (Starks 1903; Nelson 1969) (brachials of Parker 1868; radials of Swinnerton 1905a; *pièces basilaires* of Bertin 1925; radii proximals of Kampf 1961; pectoral pterygiophores of Banister MS 1967).

AB: Ascending branch of the pelvic skeleton (Bertin 1925; Nelson 1969) (postpectoral plate of Eigenmann 1887; lateral process of Berg 1907; ascending process of Regan 1909, and Hall MS 1956; *lateralteil des bauchknochens* of Leiner 1934; pelvic wings of Penczak 1965).

AP: Anterior process of the pelvic skeleton.

CL: Cleithrum (Swinnerton 1905a; Bertin and Arambourg 1958; Kampf 1961; Banister MS 1967; Nelson 1969) (clavicle of Parker 1868; Starks 1903; Bertin 1925; and Leiner 1934).

CO: Coracoid (Swinnerton 1905a; Bertin 1925; Kampf 1961; Banister MS 1967; Nelson 1969) (prae-coracoid of Parker 1868; hypocoracoid of Starks 1903, and Bertin and Arambourg 1958).

COF: Coracoid foramen.

EC: Ectocoracoid (Regan 1909: Berg 1949; and Penczak 1962) (infracleithrum of Swinnerton 1905a; Kampf 1961; and Banister MS 1967; interclavicle of Parker 1868; Bertin 1925; Leiner 1934; Hall MS 1956; and Nelson 1969; pubic bone of Eigenmann 1887, and Jordan 1888; ganoid plate ("interclavicle") of hypocoracoid of Starks 1903; *hypocoracoïde pointille* of Bertin and Arambourg 1958).

EP: Epipleural rib (Banister MS 1967, but rib of Norden 1961).

PP: Posterior process of the pelvic skeleton and ventral shield (pubic bones of Gunther 1859; Gill 1885; Berg 1907; and Hall MS 1956 (posterior pubic process of pubic bones); innominate bone of Eigenmann 1887, and Jordan 1888; pelvic plate of Regan

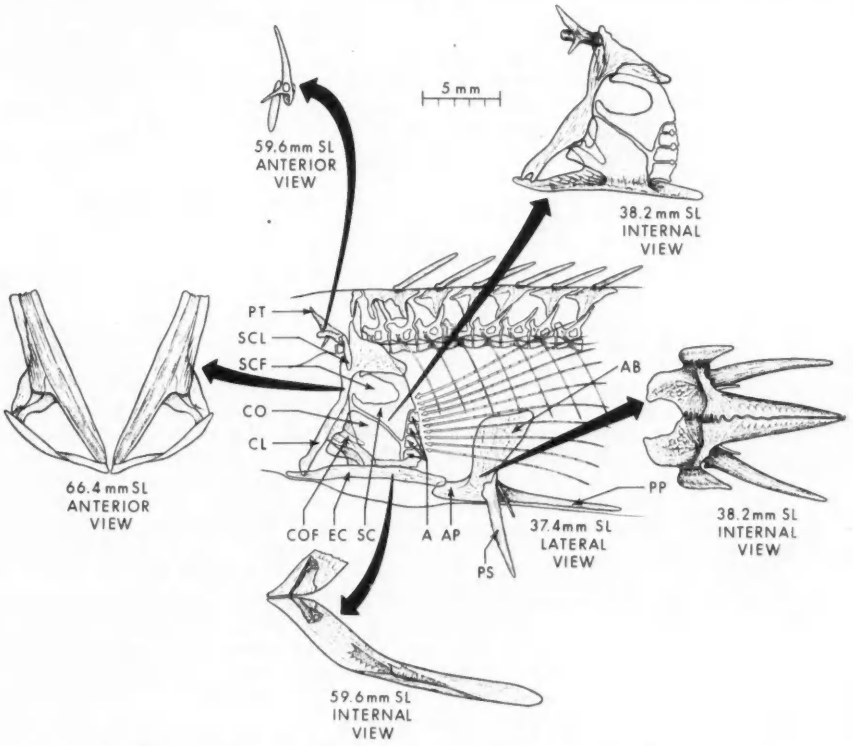

FIG. 1. Parts of the pectoral and pelvic skeleton region of *Pungitius pungitius* from Lake Superior, Wisconsin. See section under "Terminology of structures in the pectoral and pelvic skeleton region" for abbreviations.

1909; *os pelviens* of Bertin 1925; *ventraler teil des unbenannten bauchknochen* of Leiner 1934; medial plate of Penczak 1965).

PR: Pleural rib (Banister MS 1967),

PS: Pelvic spine (ventral spine of many authors).

PT: Posttemporal (Parker 1868; Starks 1903; Bertin 1925; Leiner 1934; Kampf 1961; Banister MS 1967) (supratemporal of Bertin and Arambourg 1958).

S1: First scute (plates of many authors).

SC: Scapula (Parker 1868; Swinnerton 1905a; Bertin 1925 (*scapulaire ou omoplate*); Kampf 1961 (*pars scapularis*); Banister MS 1967; Nelson 1969) (hypercoracoid of Starks 1903, and Bertin and Arambourg 1958).

SCF: Scapular foramen (various authors).

SCL: Supracleithrum (Kampf 1961; Banister MS 1967) (superclavicle and supraclavicle of Starks 1903; hypercleithrum of Bertin and Arambourg 1958).

TP: Transverse process (Banister MS 1967; but parapophysis of Norden 1961).

Pectoral and Pelvic Skeleton Region

The general anatomy, morphological variation, and development of the pectoral and pelvic skeletons of *Gasterosteus aculeatus* have been described by many authors (e.g., Cuvier and Valenciennes 1829; Parker 1868; Swinnerton 1905a, b; Starks 1903; Bertin 1925; Leiner 1934; Penczak 1964, 1965). But most of the other species in the two families have received scant attention in systematic studies, and only a superficial comparison has been made of all the species.

The pectoral skeleton in both the aulorhynchids and gasterosteids is well developed and consists of a cleithrum, scapula, coracoid, and ectocoracoid. Four actinosts, upon which all but the uppermost pectoral fin rays articulate, are also present. A posttemporal and supracleithrum are generally present and connect the pectoral skeleton to the cranium. There is no mesocoracoid or postcleithrum. The pelvic skeleton is generally well developed and highly variable, as noted below. It is never fused to any part of the pectoral skeleton. The general anatomy of this region differs in taxa comprising the seven genera (Fig. 2 and 3).

Aulichthys japonicus and *Aulorhynchus flavidus* possess a relatively similar pectoral skeleton that shows several consistent differences from the skeleton of the species placed in the Gasterosteidae. In the two aulorhynchids the large lateral flange of each cleithrum extends ventrally almost to the cleithra junction. At the ventral junction each cleithrum has a large posterior process. The ventral coracoid arm, which is expanded horizontally, attaches on a broad base to the cleithrum from the ventral margin of the cleithra flange to the tip of the ventral cleithra posterior process. A large foramen, open anteriorly, is present in the coracoid. In the gasterosteids the lateral flange and the ventral coracoid margin end well above the cleithra junction. There is no large ventral posterior process to the cleithra. The coracoid foramen is absent or very reduced. In the aulorhynchids the poorly developed external ectocoracoid is closely bound to the ventral length of the coracoid and extends as a narrow strut to the ventrolateral tip of the cleithral flange. The gasterosteid ectocoracoid is variable, as noted later.

The pelvic skeleton in aulorhynchids consists of 1 small spine and 4 soft fin rays, along with an enlarged anterior process and a reduced posterior process. In the gasterosteids the pelvic spine is much larger than the 1 or 2 soft fin rays. The posterior process exceeds or equals the length of the anterior process (except in some very young stages).

Several differences exist between the two monotypic genera of aulorhynchids. The posttemporal of *Aulichthys* has a spikelike process (the medial process) on the inner surface, which attaches to the cranium. The medial process is absent in *Aulorhynchus*. This bone carries a lateral line canal, as it does in most gasterosteids, and is continuous with the lateral scute series (but more elongate than the first scute). A supracleithrum, an elongate bone connecting the posttemporal and the cleithrum, is present in *Aulichthys* but absent in *Aulorhynchus* (Fig. 4A, E). The pelvic skeleton in *Aulorhynchus* is farther forward and has a shorter anterior process than in *Aulichthys* (Fig. 2, 4D, H). A distinct gap exists in *Aulorhynchus* between the ectocoracoid and the anterior process of the pelvic skeleton, whereas in *Aulichthys* there is a relatively close contact as a result of the forward extension of the anterior pelvic process. In *Aulorhynchus* the posterior process is median (somewhat similar to *Culaea inconstans* or to an early developmental stage in the pelvis of some other species and unlike the long lateral process in *Spinachia spinachia*) and there is a small posterolateral projection opposite the spine origin. In *Aulichthys* the posterolateral process is more distinct and the median one is very stubby. In individuals of both genera the two halves are thin and weakly sutured.

Within the gasterosteids there are obvious differences in the pectoral and pelvic skeletons (Fig. 2, 3). The posttemporal in *S. spinachia* is elongate, in line with the lateral scutes, and moderately forked with the two lobes about equal (in the very young the upper lobe is broad and elongate and the whole bone is deeper than it is long). In *Apeltes quadracus* the upper lobe is narrow and elongate

whereas the ventral lobe is very poorly developed. In the *Gasterosteus aculeatus* complex it is somewhat rhombic in shape (the posttemporal is absent in *G. wheatlandi*) whereas in the *Pungitius pungitius* complex and in *C. inconstans* it is elongate with the dorsal lobe much more extended than the ventral lobe (in *P. pungitius sinensis* the dorsal lobe is short with the whole structure tending to be rhombic). The medial posttemporal process present in *Aulichthys japonicus* is present and extends to the cranium in all gasterosteids except *C. inconstans* (in this species it is just a short knob). The posttemporal is loosely associated with the skull in *C. inconstans* but firmly fixed in the *G. aculeatus* complex (the other species are intermediate).

The supracleithrum is absent in *S. spinachia* (Fig. 5A) and *G. wheatlandi* (as it is in *Aulorhynchus flavidus*).

The head of the cleithrum has a posterior projection in *S. spinachia*, *Gasterosteus* spp., and *Pungitius* spp., but the posterior margin is rounded in *Apeltes quadracus* and *C. inconstans*.

The scapular foramen opens anteriorly and is relatively large except in *A. quadracus* (where it is reduced). A coracoid foramen is generally absent

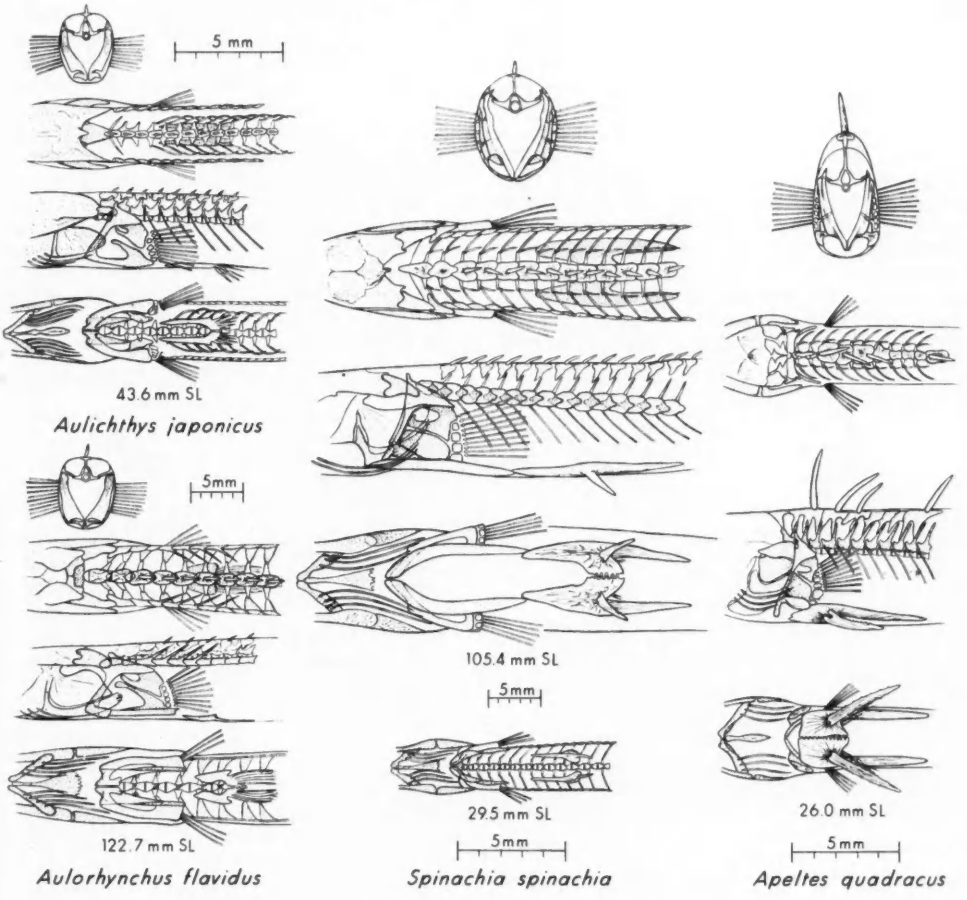

FIG. 2. Cross section (at anterior end of cleithra) and dorsal, lateral, and ventral views of *Aulichthys japonicus* (Japan), *Aulorhynchus flavidus* (Graham Island, B.C.), *Spinachia spinachia* (Isle of Man, G.B.), and *Apeltes quadracus* (Great Pond, Mass.).

but a trace of it exists in *A. quadracus* and *C. inconstans* and usually in *Pungitius* spp.

The ectocoracoid in *S. spinachia*, *Gasterosteus* spp., and *Pungitius* spp., extends anteriorly and medially to unite under the cleithral apex. There is thus an enclosed space between the ectocoracoid, coracoid, and cleithrum. In *S. spinachia* the ectocoracoid extends farther posteriorly than in members of the other two complexes (and overlaps part of the anterior process of the pelvic skeleton), but it is basically similar to that bone in *Pungitius* spp. In *Gasterosteus* spp., this structure is much heavier than in *S. spinachia* or in many specimens of *Pungitius* spp. In some Asian forms of *Pungitius*, particularly *P. platygaster*, the ectocoracoid is wide and does not extend far behind the coracoid, resembling forms of *Gasterosteus* more than the long slender ectocoracoid typical of North American *Pungitius*. *Apeltes quadracus* and *C. inconstans* lack the anterior extension of the ectocoracoid. They do have an extension over the coracoid to or toward the cleithral flange, present in the other gasterosteids, and somewhat similar to the state in the aulorhynchids. In *A. quadracus* the ectocoracoid approaches the ventral tip of the cleithrum's lateral flange and surrounds part of the pelvic skeleton with its grooved posterior margin. The ectocoracoid of *C. inconstans* abuts onto the lateral flange and overlaps the pelvic skeleton laterally with its flattened convex posterior margin. In both species the ectocoracoid is firmly bound to the coracoid except for the short posterior extension to the pelvics. I would at this time like to correct a statement made earlier (Nelson 1969 p. 2440), in referring to a statement of Swinnerton (1905a). This author concluded that the coracoid was a cartilage bone whereas the ectocoracoid was a dermal bone, which is the reverse of the statement I attributed to him.

The gasterosteid pelvic skeleton exhibits considerable diversity. The posterior process of the skeleton in *S. spinachia* and *A. quadracus* consists of two elongate prongs, which are lateral to the spines. There is no ascending branch. In members of the other three genera the pelvic skeleton consists of two juxtaposed median plates (the ventral shield), which taper posteriorly, and there is an ascending branch that generally has an incised dorsal margin in *Gasterosteus* spp., but a rounded or straight margin in members of the other two genera. The skeletons in *S. spinachia* and *A. quadracus* are markedly different in position and anatomy (Fig. 2, 5D, G, H). Of the gasterosteids, *S. spinachia* has the most posteriorly located pelvics with unique, elongate, pronglike, anterior processes, whereas *A. quadracus* possesses the most anteriorly placed pelvics without separated anterior processes. The two halves are relatively weakly sutured and thin in *S. spinachia*, whereas in *A. quadracus* the two halves are very strongly sutured and there is a

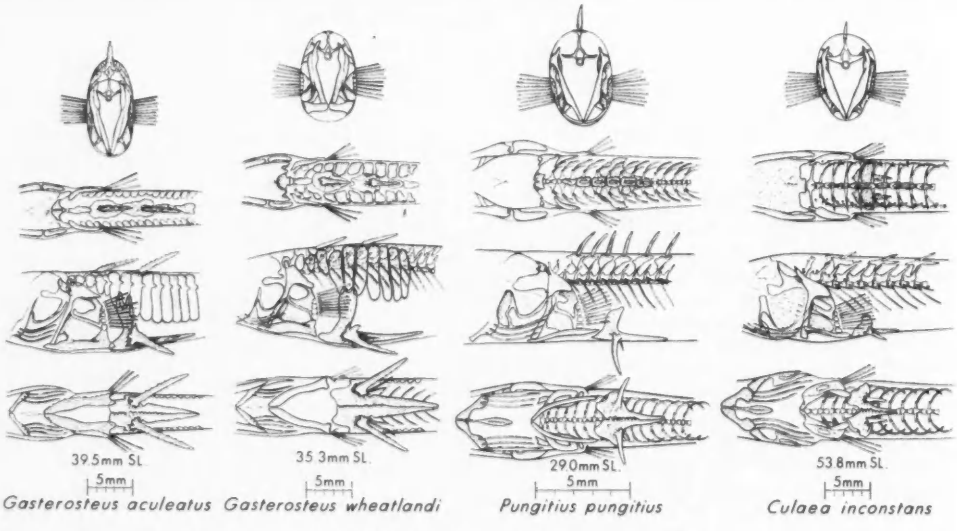

FIG. 3. Cross section (at anterior end of cleithra) and dorsal, lateral, and ventral views of *Gasterosteus aculeatus* (Maine), *Gasterosteus wheatlandi* (Maine), *Pungitius pungitius* (Crooked Lake, Indiana), and *Culaea inconstans* (Summit Lake, Alberta). The cross sections of the two species of *Gasterosteus* are at slightly different angles.

well-developed internal transverse ridge with a median foramen on the anterior surface. This ridge is relatively well developed in *Gasterosteus* spp. and *Pungitius* spp., but weak in *C. inconstans*. Of these latter three forms, the two skeleton halves are most strongly sutured together in *Gasterosteus* spp., and weakest in *C. inconstans*. The elongate separated processes in *S. spinachia* leave a deep indentation on the anterior margin, whereas in *A. quadracus*, with very short anterior processes, the anterior margin is slightly convex. In *Gasterosteus* spp. the anterior margin varies from being concave (as in *G. wheatlandi*) to straight. In *Pungitius* spp. and *C. inconstans* the anterior margin is deeply incised (concave).

The three marine or brackish water species, *S. spinachia*, *A. quadracus*, and *G. wheatlandi*, possess two pelvic fin soft rays whereas the others normally possess one soft ray. Some specimens of *Pungitius* spp. have two soft rays (as in the *P. pungitius sinensis* examined) and some are without a soft ray (Jordan and Hubbs 1925; Kobayashi 1932; Berg 1949).

The pelvic skeleton is absent in some individuals of the *G. aculeatus* complex (Hagen and McPhail 1970), the *P. pungitius* complex (Day 1878; Nelson and Atton 1971), and in *C. inconstans* (Nelson and Atton 1971).

Other Bones

The snout is elongate in *Aulichthys japonicus*, *Aulorhynchus flavidus*, and *Spinachia spinachia* (Fig. 6) with the anterior margin of the orbit lying far behind the articular-quadrate articulation. The dorsal wing (ascending process) of the premaxilla is much longer

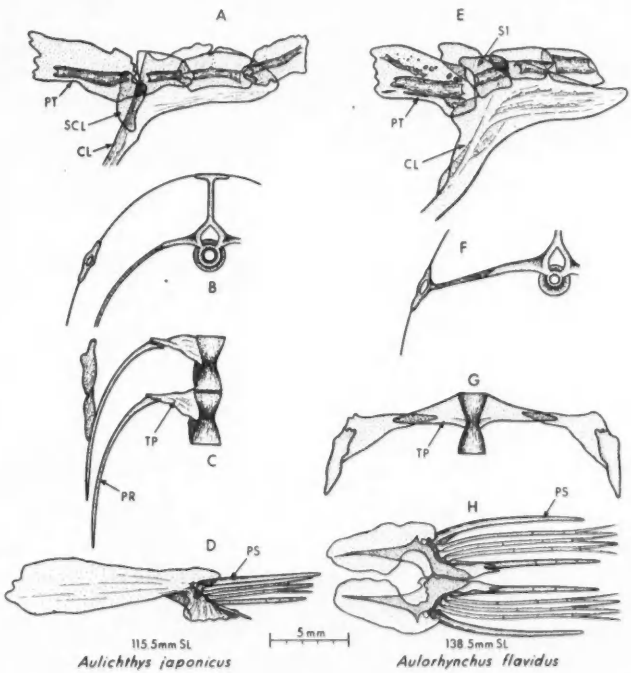

FIG. 4. Details of certain structures in *Aulichthys japonicus*: (A) lateral view of upper part of the left pectoral region; (B) cross section showing relation of pleural rib and scute on right side; (C) ventral view of vertebrae and pleural ribs from right side of midabdominal region; (D) internal view of right half of pelvic skeleton. *Aulorhynchus flavidus*: (E) lateral view of upper part of left pectoral region; (F) cross section showing relation of transverse scute process and scute on right side; (G) ventral view of vertebra and transverse scute process of midabdominal region; (H) internal view of pelvic skeleton.

than the ventral wing. In the other species the anterior orbit margin overlies, or almost overlies, the point of articulation. The dorsal wing of the premaxilla is equal to or shorter than the ventral wing. Among the former three species, *A. japonicus* has the smallest mouth. The upper jaw length is less than one-half the distance to the point of articulation (the effective mouth opening is one-third the distance to the point of articulation) and the point of articulation is far back (about one-half the distance to the orbit). In both *A. flavidus* and *S. spinachia* the upper jaw length is more than one-half the distance to the point of articulation (the effective mouth opening is more than one-half the distance to the point

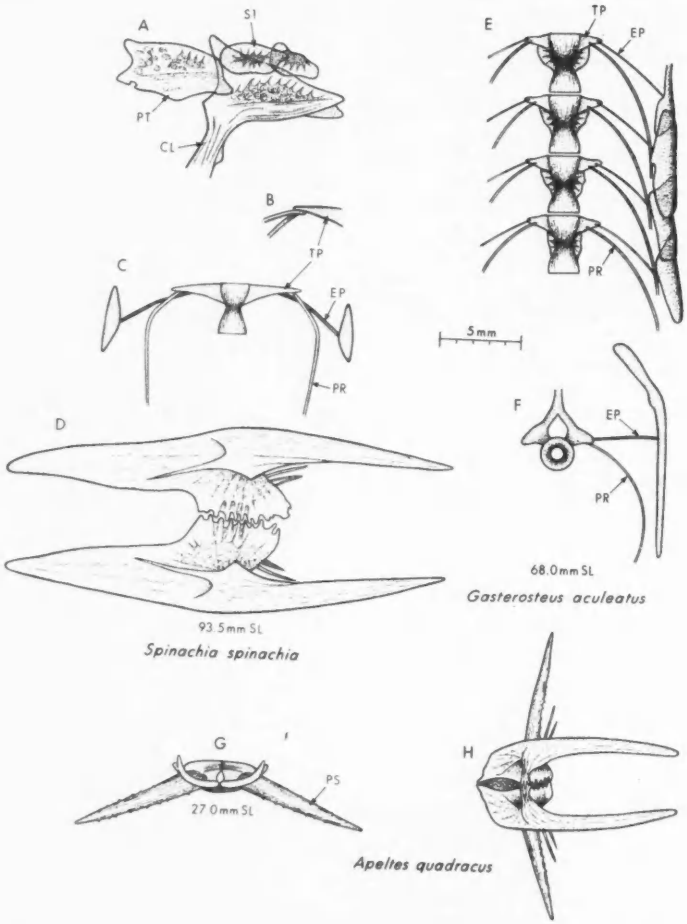

FIG. 5. Details of certain structures in *Spinachia spinachia*: (A) lateral view of upper part of left pectoral region; (B) dorsal view showing relation of ribs to transverse process on left side; (C) ventral view of vertebra and ribs of mid-abdominal region; (D) internal view of pelvic skeleton. *Gasterosteus aculeatus* from Nimpkish Lake, B.C.: (E) dorsal view of vertebrae and ribs on left side of midabdominal region; (F) cross section showing relation of ribs with scute. *Apeltes quadracus*: (G) anterior view of pelvic skeleton; (H) internal view of pelvic skeleton.

of articulation) and the point of articulation is not far back (less than one-half the distance to the orbit). *Aulichthys japonicus* has a unique dorsal projection (directed posteriorly) on the ventral premaxillary wing. In *A. flavidus* the anterior tip of the premaxilla is much more heavily ossified than in the species in the other six genera. *Spinachia spinachia* has the heaviest teeth, many have several cusps, whereas *A. japonicus* has the weakest tooth development. In gasterosteids the maxilla is curved around the posterior margin of the premaxilla, whereas in the aulorhynchids the tip is expanded posteriorly with only a small hook at the tip.

The dentary and articular of the lower jaw have dorsal processes with a distinct gap between them in the Aulorhynchidae and Gasterosteidae except for the species *S. spinachia* and *Apeltes quadracus*, where the processes are close together and partially overlap.

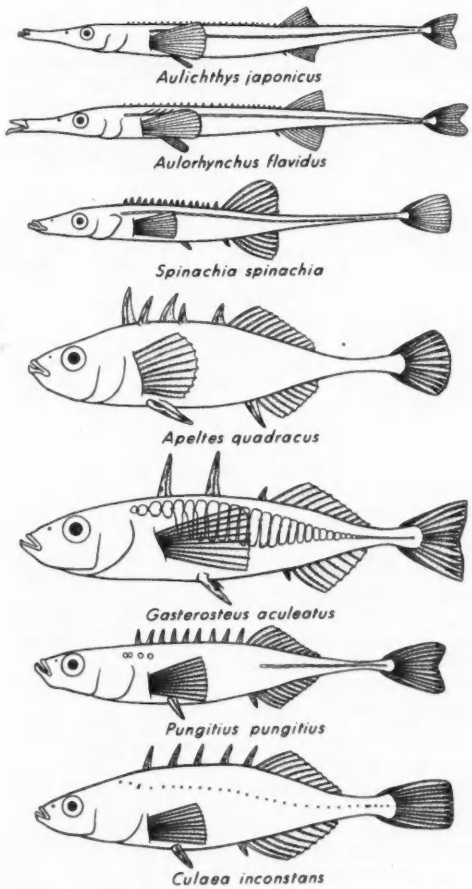

FIG. 6. General body shape of an individual of each of the seven genera.

One prominent difference between the aulorhynchids and gasterosteids is the complete enclosure of the orbit by the circumorbital bones in members of the former family and the gap in the orbit in members of the latter family. Gasterosteids have only a lachrymal and a second and third circumorbital with a space to the dermosphenotic and frontal. In *Gasterosteus* spp., this gap is relatively small due to the dorsal spike on the third circumorbital. The gap is proportionately largest in *A. quadracus*. In aulorhynchids there are tubelike fourth and fifth circumorbitals that completely enclose the orbit posteriorly. In both families the preopercle has an elongate ventral arm that underlies and abuts the ventral circumorbital bones.

Aulorhynchids have a higher number of dorsal spines, pelvic fin soft rays, lateral scutes, branchiostegal rays, caudal fin rays, and vertebrae than the gasterosteids (see next section for details). All gasterosteids possess epipleural and pleural ribs (Fig. 5B, C, E, F). *Aulichthys japonicus* possesses only pleural ribs (Fig. 4B, C) and *Aulorhynchus flavidus* appears to lack all ribs. The wide, horizontally flattened structures of this species, which extend from the scutes to the transverse processes in the abdominal region (Fig. 4F, G), perhaps perform the function of epipleural ribs, but they are firmly united with the scutes without a clear suture line and they show a gradation of short stubs on the anterior scutes and on the scutes posterior to the abdomen. They are interpreted here as being internal transverse scute processes and not modified epipleural ribs.

The hypural plate is fused in all species except in forms of the genus *Gasterosteus* where it is split into upper and lower halves. This diagnostic character, valid for all *Gasterosteus* spp., stands out in contrast to many other features, which show much intraspecific variation and overlap with other species. The hypural plate was studied embryologically by Huxley (1859) and the split is present in the fossil *Gasterosteus* described by Hay (1907).

The scute pattern and number are highly variable between the species and even within some of the species. All possess scutes on the dorsal surface from the first spine or before to the last fin ray (occasional gaps are present) and along the anal fin base. Each median scute usually has an underlying pterygiophore whether or not it bears a fin ray.

The two aulorhynchids have 52 or more overlapping lateral scutes on their elongate bodies,

whereas the gasterosteids have 42 or fewer on their proportionately shorter bodies. *Aulorhynchus flavidus* has unique dorsoposterially directed wings extending from the dorsal margin of scutes 3–6 (fewer than four wings are present in some specimens) whereas *Aulichthys japonicus* has posteriorly directed spines on its lateral scutes. The internally directed scute processes of *A. flavidus* have already been mentioned. The three elongate species, the two aulorhynchids, and *S. spinachia* (Fig. 6) have more than 12 overlapping ovoid scutes behind the scute associated with the last ray of the dorsal and anal fins (the remaining species have eight or fewer on their proportionately shorter caudal peduncle length). In *A. flavidus* and *A. japonicus* there is a gap between the lateral scutes and the median scutes (the last three to five median scutes overlie the vertebrae and are inconspicuous). However, in *S. spinachia*, unlike other gasterosteids, the posterior median and lateral scutes abut (sometimes suture), thereby enclosing an area of the caudal peduncle in bone.

Spinachia spinachia has about 41 overlapping lateral scutes whereas *Apeltes quadracus* lacks lateral scutes but has four or five small median scutes behind each fin. The *G. aculeatus* complex has 0–35 lateral scutes and usually two or three small median scutes behind the dorsal and anal fins (which end far back, close to the caudal fin). The development and large morphological variation of scutes in this complex are described by Penczak (1966), Miller and Hubbs (1969), Narver (1969), Hagen and McPhail (1970), and Igarashi (1970), and their inheritance has been studied by Münzing (1959). These workers and their numerous predecessors have found the common pattern to consist of: (*i*) a few elongate lateral scutes in the pelvic skeleton region; (*ii*) some anterior lateral scutes and some on the caudal peduncle (forming a caudal keel) with a distinct gap between; and (*iii*) a continuous row from behind the posttemporal to the tail. No population has yet been found with the scutes present only on the caudal peduncle. *Gasterosteus wheatlandi* has about 7–10 lateral scutes in the pelvic skeleton region (never on the caudal peduncle) and usually only 1 small scute behind the median fins. Members of the genus *Pungitius* have 0–34 lateral scutes. Individuals studied in this work and those studied by Igarashi (1963, 1968), McPhail (1963), Münzing (1966, 1969), and Nelson (1968a), generally have: (*i*) scutes only on the caudal peduncle (forming a keel); (*ii*) a few anterior scutes behind the posttemporal and on the caudal peduncle (forming a keel) with a scuteless area between; or (*iii*) a continuous row composed of nonoverlapping circular scutes, with a few elongate scutes in the midabdominal region (in *P. platygaster*), or composed of overlapping scutes that are again only strongly elongate in the midabdominal region and that develop a keel on the caudal peduncle (in *P. pungitius sinensis*). The *Pungitius pungitius* complex, with its longer postanal fin distance, possesses 5–8 well-developed scutes (they almost contact the lateral scutes in some individuals). *Culaea inconstans* has a row of 30–36 small circular scutes (similar to those of *P. platygaster*) along the lateral line with up to five additional scutes paired with the anterior ones (Nelson 1969). There are only 2 or 3 very small median scutes although the caudal peduncle length is similar to that of the *Pungitius pungitius* complex.

Diagnostic Features

In the following descriptions, emphasis is given to the general characteristics of the diagnostic osteological features studied. The descriptions apply only to ossified structures as seen with Alizarin; cartilaginous elements are not included. The general body shape of an individual of each of the seven genera is shown in Fig. 6.

Family Aulorhynchidae (Two Monotypic Genera)

Coracoid foramen present and open anteriorly, larger than scapular foramen; ventral arm of coracoid extending forward to junction of cleithra; ventral surface of cleithrum extending posteriorly; ectocoracoid elongate and intimately associated with the coracoid for its entire length, except for a short posterior extension; anterior process of pelvic skeleton much longer than posterior process; pelvic fin with 1 spine and 4 soft rays; circumorbital ring complete posteriorly; vertebrae 52 or more; relation of first haemal spine to anal spine pterygiophore variable; dorsal spines 24–26, very short; dorsal and anal fin soft, rays 9 or 10; caudal fin rays 13; branchiostegal rays usually 4 (3 ceratohyal and 1 epihyal); epipleurals absent, either only transverse scute processes or pleural ribs developed; posterior tip of maxilla expanded, not curved around posterior margin of premaxilla; dorsal spines, pterygiophores, and vertebrae in a 1:1:1 ratio; caudal fin distinctly forked; caudal peduncle wider than deep; gill membranes united to each other.

Species Aulichthys japonicus Gill 1863

Supracleithrum present; posttemporal with an elongate internally directed spine (the medial process), which is in contact with the skull; scapular foramen closed anteriorly in adult; pectoral fin rays 11, without basal barbs; anterior process of pelvic skeleton longer than in *Aulorhynchus*; pelvic spine originating about even with 8th vertebra and under center of posterior half of pectoral fin; first 2 pelvic fin soft rays branched, the 4th about one-half length of the longest; vertebrae 52 or 53 (usually 24 precaudal and 28 caudal — the

28th vertebra is the first one with a complete haemal canal and with a haemal spine; according to the conventional definition this species would thus usually have 27 precaudals and 25 caudals); 1st haemal spine abutting the 3rd or 4th anal fin soft ray pterygiophore; transverse process curved ventrally and not flattened; epipleurals absent, pleural ribs usually present on vertebrae 1–22; no auxiliary transverse process; lateral scutes overlapping, about 52, with lateral spine but without dorsoposterially directed wings and without inwardly directed transverse scute processes; pterygiophores above first few vertebrae bearing spines; dorsal spinelike process on ventral wing of premaxilla; jaws weakly toothed; lower jaw articulation far behind effective mouth opening (more than twice the upper jaw length).

Habitat and range: marine; Pacific Asia (Honshu and Korea).

Species Aulorhynchus flavidus Gill 1861

Supracleithrum absent; posttemporal lacking the medial process; scapular foramen open anteriorly; pectoral fin rays 10, barb present on rays on medial surface near base; anterior process of pelvic skeleton shorter than in *Aulichthys;* pelvic spine originating about even with 6th vertebra and under anterior half of pectoral fin; first 3 pelvic soft rays branched, the 4th almost equal in length to others; vertebrae 54–56 (usually 26 precaudal and 30 caudal — the last precaudal has its "auxiliary transverse process" joining the 1st haemal arch, the 1st caudal vertebra has a complete haemal arch and spine); 1st haemal spine abutting the anal spine pterygiophore; transverse process straight and flattened; epipleural and pleural ribs absent; last several precaudal vertebrae bearing short ventral riblike structures (auxiliary transverse processes) from the transverse process (these join to form the haemal arch); lateral scutes overlapping, 53–57, without lateral spines but with elongate dorsoposterially directed wings on scutes 3–6 (occasionally only on 3 and 4 or 3–5); first 28 or so scutes with transverse scute processes that join the transverse process of vertebrae 3–27; pterygiophores and dorsal scutes above 1st and 2nd vertebrae usually not bearing spines; no spinelike process on ventral wing of premaxilla (as also in gasterosteids); jaws heavily toothed; lower jaw articulation not far behind effective mouth opening (less than twice the upper jaw length).

Habitat and range: marine, Pacific North America (Alaska to California).

FAMILY GASTEROSTEIDAE (FIVE GENERA, THREE BEING MONOTYPIC)

Coracoid foramen absent (faint trace in *Apeltes, Pungitius,* and *Culaea*), scapular foramen present and open anteriorly (reduced in *Apeltes*); anterior margin of coracoid abutting cleithrum well above apex; ventral surface of cleithrum not extending posteriorly; ectocoracoid forming distinct process away from coracoid (only posteriorly in *Apeltes* and *Culaea*); anterior process of pelvic skeleton much shorter than posterior process (anterior process develops first in *Spinachia,* anterior and posterior processes about equal in length in adults); pelvic fin with 1 spine and 1 or 2 soft rays (very rarely 0 or 3); circumorbital ring incomplete posteriorly (gap wide except in *Gasterosteus* spp., where the dorsal spike of the third suborbital approaches the dermosphenotic); vertebrae 42 or fewer (the 1st caudal vertebra generally with the first complete haemal arch and haemal spine); 1st haemal spine abutting the anal spine pterygiophore; dorsal spines 16 or fewer, variable in length; dorsal and anal fin soft rays 6–14; caudal fin rays 12 (modal count for populations); branchiostegal rays 3 (2 ceratohyal and 1 epihyal), rarely 4; both epipleural and pleural ribs well developed (epipleurals usually present on all precaudal vertebrae except in *G. wheatlandi* and pleurals usually present on all precaudal vertebrae except the first two); posterior tip of maxilla not expanded, curved around posterior tip of premaxilla; spine, pterygiophore, and vertebrae ratio variable; caudal fin somewhat rounded or truncate (shallow fork in most *Pungitius*); caudal peduncle shape variable; gill membrane attachment variable.

Species Spinachia spinachia (Linnaeus) 1758

Supracleithrum absent; ectocoracoids extending forward and abutting under the junction of cleithra, forming an elongate V-shaped structure and extending posteriorly well behind the coracoid; anterior and posterior processes to the pelvic skeleton elongate and separated from their counterparts (only anterior process in very young), no transverse connecting ridge; ascending branch absent; pelvic fin with 1 smooth spine and 2 soft rays; pelvic spines posteriorly located, originating about even with 11th or 12th vertebra, near tip of pectoral fin; vertebrae 40–42 (usually 18 precaudal and 23 caudal); hypural plate solid; dorsal spines 15 (rarely 14 or 16), divergent from the midline; dorsal fin soft rays usually 6 or 7; anal fin soft rays usually 6 or 7; lateral scutes overlapping, subcircular, about 41; dorsal spines, pterygiophores, and vertebrae in a 1:1:1 ratio (except for anterior pterygiophore, which does not bear a spine); caudal peduncle wider than deep; gill membranes united to each other.

Habitat and range: marine; Atlantic Europe (Norway to France).

Species Apeltes quadracus (Mitchill) 1815

Supracleithrum present; ectocoracoid very small, closely bound to the coracoid except for a short posterior portion and joined to the lateral flange of the cleithrum, not extending forward to abut the opposite member below the apex of the cleithra; pelvic skeleton lacking distinct anterior process, the posterior process being elongate and separated from its counterpart; heavy transverse ridge on anterior part of main body of pelvis with a medial foramen on anterodorsal surface; ascending branch absent; pelvic fin with 1 serrated spine and 2 soft rays (one specimen had 3 soft rays in the left fin); pelvic spines anteriorly located, originating about even with 4th vertebra, under anterior half of pectoral fin;

vertebrae 30–33 (usually 11 precaudal and 20 caudal); hypural plate solid; dorsal spines 4 or 5 (very rarely 3 or 6), divergent from the midline; dorsal fin soft rays usually 10–13; anal fin soft rays usually 8–10; lateral line scutes absent; dorsal spines fewer than pterygiophores and dorsal scutes, pterygiophores and vertebrae in 1:1 ratio; caudal peduncle deeper than wide, subcircular in section; gill membranes joined to isthmus.

Habitat and range: usually marine and brackish water, rarely fresh water; Atlantic North America (Newfoundland to Virginia).

Genus Gasterosteus

Ectocoracoids extending forward and abutting under the junction of cleithra, forming a heavy V-shaped structure; pelvic skeleton with wide, median, juxtaposed posterior processes forming a ventral shield; ascending branch present, generally with an incised dorsal margin; pelvic spine serrated (rarely smooth) and originating about even with 5th or 6th vertebra and under middle of pectoral fin; hypural plate with a deep central split; dorsal spines usually 3 (2 long and 1 short), rarely 1, 2, or 4 reported in *G. aculeatus*, straight; dorsal spines fewer than pterygiophores and dorsal scutes, pterygiophores fewer than vertebrae; caudal peduncle variable, never as depressed as in *Spinachia*, sometimes as in *Culaea;* gill membranes joined to isthmus.

Species complex Gasterosteus aculeatus

Posttemporal and supracleithrum present; pelvic fin with 1 soft ray; pelvic spine base usually without a ventral cusp (localities are known in British Columbia with individuals that lack the pelvic skeleton); vertebrae 29–33 (most commonly 32, with 14 precaudals and 18 caudals); first haemal arch and spine usually on vertebrae 13–15; transverse process not reduced; dorsal fin soft rays 10–14 (very rarely 9); anal fin soft rays 8–11 (very rarely 6 or 7); lateral scutes 0–35; epipleural ribs commonly on vertebrae 1–13; pleural ribs commonly on vertebrae 3–14.

Habitat and range: anadromous and fresh water; Atlantic and Pacific coastal areas of northern North America (south to southern California and New Jersey) and Eurasia, throughout much of Europe, along part of the Arctic coastline.

Species Gasterosteus wheatlandi Putnam 1867

Posttemporal and supracleithrum absent (a small scute occasionally lies in the position normally occupied by the posttemporal); pelvic fin with 2 soft rays (very rarely 1 or 3 on one side); pelvic spine base with a well-developed ventral cusp; vertebrae usually 28 (12 precaudals and 16 caudals); first haemal arch usually on vertebra 10 and first haemal spine on vertebra 13; transverse process reduced; dorsal fin soft rays 9–11; anal fin soft rays 6–9; lateral scutes 6–11 confined to pelvic area (caudal peduncle keel never present); epipleural ribs commonly on vertebrae 1–8; pleural ribs commonly on vertebrae 3–12.

Habitat and range: marine (known in fresh water from Newfoundland (Scott and Crossman 1964) and Quebec (McAllister 1960); Atlantic North America (Newfoundland to Massachusetts).

Species complex Pungitius pungitius

Supracleithrum present; ectocoracoids extending forward and abutting under the junction of cleithra, forming an elongate V-shaped structure; pelvic skeleton with median juxtaposed posterior processes (wide to narrow); ascending branch present with a rounded or straight dorsal margin (localities are known in Alberta and Ireland with individuals that lack the pelvic skeleton — it is much reduced in *P. timensis* of Japan); pelvic fin with 1 serrated or smooth spine and 1 or 2 soft rays (rarely 0); pelvic spines originating about even with 6th or 7th vertebra and under middle area of pectoral fin; vertebrae 31–35 (usually 14 precaudals and 18 or 19 caudals; *P. platygaster* commonly has 13 precaudals and 16 caudals); hypural plate solid; dorsal spines 7–12 (usually 8–10, reported much lower in Ireland by Ure 1962), divergent from the midline; dorsal fin soft rays 9–12; anal fin soft rays 7–11 (*P. platygaster* commonly has as few as 7 dorsal soft rays and 6 anal soft rays); lateral scutes variable in shape, 0–34, sometimes confined to caudal peduncle or to caudal peduncle and behind pectoral skeleton, or continuous; dorsal spines and pterygiophores in 1:1 ratio (usually 2 pterygiophores at anterior end that do not bear spines), pterygiophores slightly fewer than vertebrae; caudal peduncle variable (usually wider than deep but in *P. platygaster* it is deeper than wide); gill membranes united to each other.

Habitat and range: anadromous and fresh water; Atlantic, Pacific, and arctic coastal areas of North America (south to Alaska and New Jersey) and Eurasia (south to Greece and Japan) and across much of above continental areas.

Species Culaea inconstans (Kirtland) 1841

Supracleithrum present; ectocoracoid short, not extending forward to cleithrum; pelvic skeleton with narrow, median, juxtaposed posterior processes; ascending branch present with a rounded or straight dorsal margin) (localities are known in Alberta and Saskatchewan with individuals that lack the pelvic skeleton); pelvic fin with 1 smooth spine and 1 soft ray; pelvic spine originating about even with 6th vertebra and under middle area of pectoral fin; vertebrae 30–35 (usually 14 precaudals and 18 caudals); hypural plate solid; dorsal spines 4–7 (very rarely fewer, usually 5 or 6), straight; dorsal fin soft rays 8–13; anal fin soft rays 7–12; lateral scutes circular, nonoverlapping, 30–36; dorsal spines and pterygiophores in 1:1 ratio (usually 2 pterygiophores at anterior end that do not bear spines), pterygiophores fewer than vertebrae; caudal peduncle deeper than wide; gill membranes united to each other.

Habitat and range: fresh water (very rarely brackish); North America (from British Columbia and southwestern Northwest Territories to New Brunswick and New York.)

Discussion and Conclusions

The gasterosteids are generally postulated to be derived from the aulorhynchid line, which is thought to be relatively generalized and primitive. Gill (1863, 1885) suggested that of the various sticklebacks, *Spinachia spinachia* is the closest link between the two families. This has received general support and acceptance from morphological, ecological, and behavioural studies (Bertin 1925; Hall MS 1956; Reisman and Cade 1967). Leiner (1934) regarded *Apeltes quadracus* as the most primitive gasterosteid but, like other workers, considered that *S. spinachia* and *A. quadracus* were relatively close, with *Gasterosteus aculeatus*, *Pungitius pungitius*, and *Culaea inconstans* as derivatives. The interrelations of the latter three taxa have been viewed differently but all phylogenetic schemes and even the earliest classifications place them relatively close together. For example, after the initial grouping of all sticklebacks into the Linnaean genus *Gasterosteus*, Jordan and Gilbert (1883) retained members of *Gasterosteus*, *Pungitius*, and *Culaea* in the genus *Gasterosteus* whereas *S. spinachia* and *A. quadracus* were placed in monotypic genera. Gill (1885) maintained the same relationship but placed the five genera in three subfamilies, Apeltinae, Gasterosteinae, and Spinachiinae.

The present study, in examining more anatomical characters on a comparative basis than heretofore, suggests that our understanding of the phylogenetic relationships is more insecure than previously thought. Workers such as Bertin and Leiner did not find that their characters taken together gave clear conclusions on affinities. In order to present a "neat" phylogeny they regarded some characters as better indicators of affinities than others in somewhat of the fashion so ably described by Mayr (1969). The paucity of stickleback fossils, however, makes criteria of constancy and consistency impossible to establish and places an undesirably large burden of evidence on new finds for those workers bent on deriving extant species from whatever extinct species are known.

This study did not find less incongruency than Bertin and Leiner, but more. Most extant named species in the two families are highly differentiated, as reflected in the placing of five species in monotypic genera. Many species possess unique characters (see preceding sections), and many different combinations of species or genera pairs or triplets can be picked that share characteristics to the exclusion of all others.

The details of the pectoral and pelvic skeletons reveal much diversity between the genera and show similarity within each of the two families but do little to reveal a consistent phylogeny and offer little support to the contention that either *S. spinachia* or *A. quadracus* is an evolutionary intermediate or even that the two are close to one another. The very small coracoid foramen in *A. quadracus*, *Pungitius* spp., and *C. inconstans* might suggest that these three taxa are between the aulorhynchids, with their well-developed coracoid foramen, and the remaining gasterosteids, which lack the foramen. The ectocoracoid is similar in *S. spinachia* and *Pungitius* spp., whereas *A. quadracus* and *C. inconstans* share a relatively similar ectocoracoid that, perhaps of all the gasterosteids, approaches the aulorhynchid line closest. In the pelvic skeleton, the possession of elongate anterior processes and separated posterior processes in the aulorhynchids and in *S. spinachia* cannot be considered as indicating a common ancestry. The structures are different in shape and relative position. There is no reason to believe that the pelvic skeleton of *S. spinachia* is more likely to be derived from the aulorhynchid skeleton than the pelvics of *C. inconstans*. Similarly, the pelvic skeletons in *S. spinachia* and *A. quadracus* are very different. The only similarity, the possession of separate posterior processes, hardly denotes common ancestry. The value to be attached to the common possession of two pelvic fin soft rays in *S. spinachia and A. quadracus* in indicating affinity and evolutionary intermediacy between the higher number in aulorhynchids and the lower number in most gasterosteids must also be held in doubt. *Gasterosteus wheatlandi* also possesses two soft rays whereas *Pungitius* spp. is quite variable. There is no reason to believe it to be a conservative character (nor does there seem to be any sound reason for assuming that characters of unknown function are nonadaptive and conservative with much systematic value). The pelvic skeletons in forms in the genera *Gasterosteus*, *Pungitius*, and *Culaea* are similar and do imply common ancestry for this character.

The absence of bones may mean less than their comparative anatomy when present. The loss of the pelvic skeleton in *G. aculeatus*, *P. pungitius*, and *C. inconstans* is a local phenomenon. Individuals of populations within each species that lose the skeleton are almost certainly derived from a population with the pelvics. Similarly, *G. wheatlandi* almost certainly shares a common ancestry with *G. aculeatus* but the former is unique in the absence of the posttemporal. The lack of this strengthening support may be related to lower fanning activity than in *G. aculeatus* as observed by McInerney (1969). Also, the common absence of a supracleithrum in *Aulorhynchus flavidus* and *S. spinachia*

should not be construed to be of systematic value in indicating relationship at present.

In addition to the lack of concordance in the various characters, different trends are apparent. Within the gasterosteids, the order of genera proceeding from dependance on sea water with no fresh water penetration to dependance on fresh water with the greatest fresh water penetration is *Spinachia*, *Apeltes*, *Gasterosteus*, *Pungitius*, and *Culaea* (Nelson 1968b); aulorhynchids would rank even with *Spinachia*. Scute development (*Spinachia*, *Gasterosteus*, *Pungitius*, *Culaea*, and *Apeltes*), dorsal spine number (*Spinachia*, *Pungitius*, *Culaea*, *Apeltes*, and *Gasterosteus*), selected behavioural traits as studied by Hall (MS 1956) (*Spinachia*, *Apeltes*, *Culaea*, *Pungitius*, and *Gasterosteus*) etc., all give different trends. Little support can be marshalled to substantiate any attempt to polarize these trends to indicate direction in their evolution by favouring a few characters as being the only indicators of affinity. However, I accept the arguments of Hall (MS 1956) and others that *S. spinachia* comes closest to representing the ancestral stickleback but it has too many differences with the aulorhynchids to be considered an evolutionary intermediate.

The two families Aulorhynchidae and Gasterosteidae are distinct, but nevertheless they have probably had a common origin. They appear to be phylogenetically closest to one another and Banister's (1970) work shows that the Indostomidae do not belong in the aulorhynchid–gasterosteid line as tentatively held by Berg (1940) and Greenwood et al. (1966). The problem of lumping, as raised by Gill (1885), Starks (1903), Limbaugh (1962), and Reisman and Cade (1967), remains a subjective one but the many differences with no transition in extant species makes it desirable to leave the families separate.

The interrelations of Gill's subfamilies, considered here as natural units, and the two aulorhynchid species are considered too poorly known to speculate upon. It seems wisest to regard the many differences as indicative of much divergence with no extant species approaching the ancestral form in its general characteristics. The evidence for evolution is overwhelming; the problems that beset us in reconstructing its pathway, however, are immense.

At best, with this mosaic pattern, one could only discuss the evolution of various characters, but little objective reasoning can be put forth to establish which are the primitive and advanced states. To know the phylogeny one must know the primitive characters, but to know this demands the unlikely prospect of obtaining a complete fossil record (from which the phylogeny would be known anyway).

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NOTES

A Versatile Fortran Computer Program for the Capture–Recapture Stochastic Model of G. M. Jolly

E. G. WHITE

Tussock Grasslands and Mountain Lands Institute
P.O. Box 56, Lincoln College, Christchurch, New Zealand

WHITE, E. G. 1971. A versatile Fortran computer program for the capture–recapture stochastic model of G. M. Jolly. J. Fish. Res. Bd. Canada 28: 443–445.

A Fortran program written for an IBM 1130 computer is described for analyses of capture–recapture data using Jolly's 1965 stochastic model. The program versatility permits ready analysis of most forms of data and also provides for selections in the organisation of analyses.

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JOLLY (1965) and Seber (1965) independently formulated a very general capture–recapture theory that Cormack (1968) suggests be known as the Jolly–Seber method. Both models provide identical estimates of population parameters but Jolly's model permits the removal of animals from a sample and is therefore slightly more general. A Fortran program, written for an IBM 1130 computer, is described for Jolly's model. A program listing and write-up are given by White (1971).

DESCRIPTION OF THE PROGRAM

The program is in five sections that store the raw data and then organise any given selection of data into trellis diagrams and a table of derived estimates. The trellis diagrams and table of estimates are printed in the format of Jolly's tables 2–4, with additions as described below.

The versatility of the program extends to the following functions:

1. Data may be used from sampling programs *with or without* separate organisation for future recaptures, an alternative described by Jolly. Regardless of whether all data belong to separate release samples or whether recapture data are from any number of intermediate samples or a continuous recapture program, the appropriate analyses are performed and the tabulations printed.

2. Organisation and analysis of data can be carried out for any selection of consecutive data from a longer time sequence of data. Thereby any section of the data can be analysed separately starting at any release time a ($1 \leqslant a < b$) and stopping at any subsequent release *or* recapture time b.

3. The immigration of *marked* (as well as unmarked) animals into the population is accounted for by classifying marked immigrants as new releases when first captured after immigrating. Such immigration may occur in space or time. Hence, when $a > 1$ in section 2 above, those animals that are marked before a are at first "immigrants" and classified as new releases when subsequently recaptured and released.

4. Individual animals can be assigned as many as four attributes in order to classify and analyse the data pertaining to any given subpopulations. Thus animals might be identified by site, species, age, and sex, and various subpopulations then analysed separately. The selected sequence of analyses proceeds automatically.

5. Certain restrictions can be placed upon the analysis of data. For example, in the analyses of different class combinations above, data of each given combination are tested to check whether sufficient of the samples have an acceptable number of recaptures to proceed with analyses.

6. Both marked and nonmarked animals may be removed from any sample.

The program expands Jolly's tables to include the following in the print-out:

1. The number of immigrants in each sample, as indicated by the first column in Table 1. No distinction can be made between immigrants in time and space but both can occur together *only* when $a > 1$ and the combination of data applies to a restricted range within the experimental area.

Printed in Canada (J1961)

TABLE 1. Trellis diagram of n_{ij} values, showing the number of animals from sample size n that were captured in the ith sample and last captured in the jth sample ($32 \leqslant j \leqslant i-1 \leqslant 54$) (Jolly's nomenclature). I_i = number of immigrants in sample i; s_i = number released from ith sample after marking; R_i = number of the s_i animals that are caught subsequently; sample 32 taken September 22–24, 1969 (spring), sample 55 taken May 6–7, 1970 (autumn).

I_i	n_i	s_i																							
40	149	149	32																						
16	47	47	31	33																					
17	31	31	8	6	34																				
7	116	116	8	8	10	35																			
5	24	24	6	2	1	10	36																		
2	10	10	1	1	1	4	1	37																	
2	8	8	3	1	0	1	1	0	38																
5	85	85	3	0	0	0	0	1	0	39															
2	24	24	0	0	0	3	0	0	0	19	40														
5	64	63	0	0	0	0	1	0	0	3	2	41													
3	22	22	0	0	0	1	0	0	0	4	2	12	42												
3	84	84	0	0	0	0	0	0	0	2	1	4	3	43											
1	8	8	0	0	0	0	0	0	0	2	1	0	1	3	44										
3	103	103	0	0	0	0	0	0	0	0	0	2	0	9	1	45									
3	21	21	0	0	0	0	0	1	0	0	0	2	1	2	2	10	46								
3	145	145	0	0	0	0	0	0	1	2	0	0	1	3	1	8	5	47							
2	37	37	0	0	0	0	0	0	0	0	0	0	1	1	0	5	3	25	48						
5	139	138	0	0	0	0	0	0	0	0	0	1	0	2	0	6	1	14	8	49					
4	65	65	0	1	0	0	0	1	0	0	1	1	1	1	0	4	2	13	4	32	50				
10	157	157	1	0	0	1	0	0	0	0	0	0	0	0	0	3	0	9	2	10	19	51			
8	70	70	0	0	0	0	0	0	0	0	0	3	0	0	0	0	3	5	3	9	8	31	52		
6	111	111	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	1	0	1	3	16	18	53	
7	70	70	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0	4	2	7	11	36	54
4	78	78	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	1	1	1	1	2	8	22	55
	R_i		61	19	12	21	3	3	1	33	7	25	8	23	5	37	14	68	18	57	33	55	31	44	22

2. An estimate of the probability of capture at time i (Jolly's notation is used hereon) is included, as indicated by the third column in Table 2. This estimate is useful to check for homogeneity between subpopulations, as in the case where different class combinations of data are analysed separately and compared.

3. Asterisks are printed in Table 2 to mark those estimates that cannot be calculated when there are intermediate recapture samples that do not include unmarked animals.

The only major limitation of the program is that it is designed for data in which all animals are individually distinguished rather than grouped collectively by mark status or recapture history. Such individual identification is essential to distinguish immigrants and to thereby carry out some of the select groupings of data made possible in the program. However, in analyses not involving immigration, collective data *can* be adapted by ascribing suitable arbitrary numbers to all animals in accord with the correct tallies for each n_{ij} value in Table 1. Alternatively, such data could be directly adapted to the section of the program that calculates the population estimates listed in Table 2.

EXAMPLE

The two acridid grasshoppers *Paprides nitidus* Hutton and *Sigaus australis* (Hutton) are common alpine species in the Craigieburn Range in Canterbury, New Zealand. In a capture-recapture study with intermediate recapture samples, data from individually marked adults were analysed according to species, sex, and site combinations. Tables 1 and 2 show computed results for data

TABLE 2. Population estimates and variances (Jolly's nomenclature). α_i = proportion of recaptured animals in sample i; p_i = probability of an animal alive at time i being caught in the ith sample; M_i = total number of released animals bearing marks that are in the population at time i; N_i = total number of animals in the population when the ith sample is captured; B_i = number of new animals joining the population in the interval between the release time i and the subsequent release time and still alive at the subsequent release time; φ_i = probability that an animal alive at the moment of release of the ith sample will survive until the time of capture of the $i + 1$th sample; * means that no estimate is possible from a recapture sample.

i	$\hat{\alpha}_i$	$\hat{p}_i$	$\hat{M}_i$	$\hat{N}_i$	$(V(\hat{N}_i))^{\frac{1}{2}}$	$(V(\hat{N}_i \mid N_i)^{\frac{1}{2}}$	$\hat{B}_i$	$(V(\hat{B}_i))^{\frac{1}{2}}$	$\hat{\varphi}_i$	$(V(\hat{\varphi}_i))^{\frac{1}{2}}$	$\left(V(\hat{\varphi}_i) - \frac{\hat{\varphi}_i^2(1-\hat{\varphi}_i)}{\hat{M}_{i+1}}\right)^{\frac{1}{2}}$
32	–	–	0	–	–	–	–	–	0.706	0.119	0.113
33	*	*	105.2	*	*	*	*	*	0.861	0.231	0.229
34	*	*	104.4	*	*	*	*	*	1.170	0.349	0.351
35	0.224	0.183	142.0	633.5	175.5	175.1	652.9	373.8	0.875	0.472	0.472
36	*	*	203.0	*	*	*	*	*	0.327	0.226	0.224
37	*	*	68.0	*	*	*	*	*	1.800	1.836	1.841
38	*	*	126.0	*	*	*	*	*	0.273	0.258	0.255
39	0.047	0.115	34.9	741.8	382.3	382.2	291.9	339.0	0.870	0.257	0.255
40	*	*	100.9	*	*	*	*	*	0.646	0.227	0.222
41	0.094	0.090	66.5	709.1	299.7	299.3	317.7	365.8	0.822	0.233	0.230
42	*	*	101.5	*	*	*	*	*	1.074	0.356	0.357
43	0.119	0.089	112.3	943.0	337.7	337.3	412.3	314.5	0.416	0.127	0.122
44	*	*	77.4	*	*	*	*	*	1.467	0.443	0.452
45	0.117	0.104	115.0	987.1	302.7	302.2	282.3	227.5	0.495	0.091	0.084
46	*	*	102.0	*	*	*	*	*	1.195	0.208	0.214
47	0.145	0.167	125.5	866.4	188.4	187.5	177.9	180.1	0.816	0.137	0.134
48	*	*	203.6	*	*	*	*	*	0.957	0.184	0.183
49	0.230	0.163	196.6	854.1	157.3	156.1	141.0	111.4	0.618	0.083	0.078
50	*	*	187.1	*	*	*	*	*	1.012	0.157	0.157
51	0.287	0.233	193.4	674.9	111.4	109.8	77.7	51.1	0.536	0.076	0.071
52	*	*	163.6	*	*	*	*	*	0.753	0.124	0.119
53	0.369	0.317	129.3	350.0	57.2	55.3	*	*	0.572	0.090	0.083
54	*	*	113.9	*	*	*	–	–	–	–	–
55	0.487	–	–	–	–	–	–	–	–	–	–

obtained during the 1969–70 season and analysed as a joint combination of both species and both sexes within a limited range of the experimental area. Since this analysis commenced with the 32nd sample, many previously marked animals (from the 1968–69 season) that were already present within the range were recaptured and initially classified as immigrants, as reflected by the first high numbers in column 1 of Table 1. This form of "immigration" quickly diminishes and is supplemented by the continuing immigration of marked animals from outside the range, thereby accounting for the steady small numbers of immigrants throughout the remainder of the sampling period. Other modifications to the format of Jolly's tables have already been described above. The trellis table of accumulative values (Jolly's table 3) is unmodified by the program and therefore omitted from this example.

Acknowledgments — The author gratefully acknowledges the guidance of Dr G. A. F. Seber in clarifying aspects of capture–recapture theory, and Miss E. E. Emerson for her invaluable assistance in the writing of the program.

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Electronic Processing of Acoustical Data for Fishery Research[1]

WILLIAM H. LENARZ[2] AND JAMES H. GREEN[3]

Fisheries Research Institute and Division of Marine Resources
University of Washington, Seattle, Wash., USA

LENARZ, W. H., AND J. H. GREEN. 1971. Electronic processing of acoustical data for fishery research. J. Fish. Res. Bd. Canada 28: 446–447.

A system for processing acoustical data is described. Acoustical data are recorded on magnetic tape in analog form in the field. The data are converted to digital form and analyzed with the aid of a digital computer. The system provides investigators with considerably more information than is available from paper records now in common use.

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ACOUSTICAL techniques are being used increasingly for the study of fish behavior (Narver 1970; Finnell and Reed 1969) and for estimation of the size of fish stocks (Cushing 1968). Usually, acoustical returns from fish are recorded on paper charts and then counted. Counting fish targets is a subjective and tedious process and, consequently, several electronic devices have been developed to estimate target densities. An example is the echo integrator that was developed by Lahore and Lytle (1970.) These devices have been used successfully in the field (Thorne and Woodey 1970), but they are expensive and add to the complexities of field work. Another approach to estimating target densities is described in this paper: acoustical data are recorded on magnetic tape and are automatically processed by means of a digital computer.

Returning signals from a Simrad ER 4 or EK 38A echo sounder are recorded by a Sony 560 D tape recorder in analog form onto a standard four-track magnetic tape. Echo signals are recorded on one channel, and a timing pulse is recorded on the other channel as a pulse of sound is emitted by the sounder. The echo signal is recorded as a 6-kHz sine wave, 100% amplitude modulated by the returning echo from the sounder. The operator of the sounder records a vocal log at the beginning of each set of soundings onto the tape.

The data are converted from analog to digital form by a unique analog-to-digital converter that the Department of Atmospheric Sciences of the University of Washington constructed. We developed electronic circuitry to reduce the data to a more efficient form during the digitizing process.

Figure 1 illustrates the nature of the signal from the time that it is emitted by the sounder until it has been converted to digital form. The signal from the tape recorder is passed through a full-wave rectifier. This device can be expressed mathematically by the equation

$$V_{out} = |V_{in}|, \qquad (1)$$

where V_{out} = output voltage, and V_{in} = input voltage. Then the timing pulse is subtracted from the signal so that all information is stored in one series of data. It is simple to distinguish the negative timing pulse from the remainder of the data. Next, the signal is passed through a 1-kHz, low-pass filter. We choose a 1-kHz cut-off frequency because the length of the pulse of sound transmitted by the sounder is about 1 mv-sec, thus there should be no significant variations at frequencies higher than 1 kHz left in the return signal.

Much of the signal contains data that are not interesting to us. We are never interested in echos returning from depths greater than 100 m. In many instances, only the upper 30–40 m are of interest. We developed a timing gate to eliminate the unnecessary data. This device allows us to reduce the amount of data by more than 80%. Since returns from adjacent pulses of sound are highly correlated, it is not always necessary to sample every pulse to obtain desired results. Therefore, we developed a device to allow us to sample every first, second, third, or fourth pulse. The data are digitized then at a 2-kHz rate and recorded in binary form on magnetic tape.

A computer program was written in the Fortran language for a Control Data Corporation 6400 computer to perform preliminary analyses of the data. Since an enormous amount of data is involved (an average of 18,000 observations per minute

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[1]Contribution No. 339, College of Fisheries, University of Washington.

[2]Present address: Bureau of Commerical Fisheries, La Jolla, California.

[3]Present address: Division of Marine Resources, University of Washington, Seattle, Wash.

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of sounding), particular attention was devoted toward developing an efficient program. The data are packed five observations per 60-bit computer word. Most of the calculations are made in integer mode.

Several calculations are made by this program. During the digitizing process, a record of data is created without movement of the analog tape. This group of data represents the noise level of the system. The average noise level is calculated and subtracted from each observation. Changes in gain of the digitizing system are estimated from the timing pulse. The gain is standardized by multiplication of each observation by a standard value divided by the minimum value of the timing pulse within a specified time interval. The user is allowed to supply a TVG (time varying gain) function, which corrects for increased attenuation of the signal with depth. Voltage and voltage squared are summed at user-specified intervals of time and depth. Statistical frequency distributions of target strengths are produced at specified intervals of time, target strength, and depth.

The calculations made by this program represent a minimal analysis of the data. We anticipate that the availability of acoustical returns from fish targets in digital form will facilitate the development of methods of target classification. Figure 2 is a three-dimensional plot of returns from a target in Iliamna Lake, Alaska. There is considerably more information available to investigators than would be available from paper recordings now in common use.

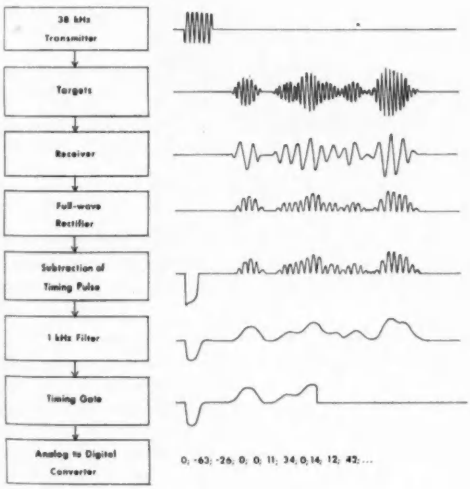

FIG. 1. Flow chart of acoustic signal from emission by sounder to conversion to digital form.

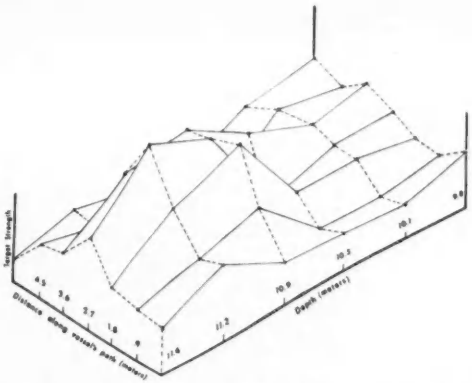

FIG. 2. Three-dimensional plot of echo returns from a target in Iliamna Lake, Alaska.

A detailed description of the electronic circuitry and computer software may be obtained from the Fisheries Data Analysis Center, Fisheries Research Institute, Univeristy of Washington, Seattle, Wash. 98105, USA.

Acknowledgments — This research was supported in part by NSF Grant GH-40 under the Sea Grant Program.

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Subcutaneous Fibroma in Lake Trout (*Salvelinus namaycush*) in Quebec

E. BROUGHTON AND L. P. E. CHOQUETTE

Canadian Wildlife Service
Department of Indian Affairs and Northern Development, Ottawa, Ont.

BROUGHTON, E., AND L. P. E. CHOQUETTE. 1971. Subcutaneous fibroma in lake trout (*Salvelinus namaycush*) in Quebec. J. Fish. Res. Bd. Canada 28: 448–449.

Histological examination of a subcutaneous tumour in each of two lake trout (*Salvelinus namaycush* (Walbaum)) from two lakes in the province of Quebec showed it to be a fibroma. This is the first report of such a neoplasm in lake trout.

BROUGHTON, E., AND L. P. E. CHOQUETTE. 1971. Subcutaneous fibroma in lake trout (*Salvelinus namaycush*) in Quebec. J. Fish. Res. Bd. Canada 28: 448–449.

L'examen histologique d'une tumeur sous-cutanée chez chacune de deux truites de lac (*Salvelinus namaycush* (Walbaum)) prises dans deux lacs au Québec révèle un fibrome. C'est la première fois que l'on rapporte la découverte d'un tel néoplasme chez la truite de lac.

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NEOPLASMS in freshwater fishes have been the subject of reviews by Schlumberger and Lucké (1948), Nigrelli (1953), and Wellings (1969). In this note, we deal with subcutaneous fibroma in two lake trout (*Salvelinus namaycush* (Walbaum)) caught by sportsmen in the province of Quebec, Canada, and submitted to our laboratory by the Quebec Fish and Game Branch.

One lake trout, 57 cm long and weighing 7½ lb, was taken in Lac Trente-et-un-Milles, Gatineau County, in June 1968. Scale examination showed it to be 8 years old. It exhibited a large swelling on the right side, just lateral to the dorsal fin (Fig. 1), that was firm, but pitted under marked pressure. The skin over the swelling was reflected, and the encapsulated tissue mass enucleated by blunt dissection. The tumour was nearly round, 11 cm in diameter, and weighed 750 g. A large gauge needle and a syringe were used to aspirate from the tissue mass 120 ml of pale, reddish-brown serous fluid.

The other trout, 62 cm long and weighing 8 lb, and also 8 years old, was caught in Lac McCarthy, Papineau County, in September 1969. The tumour on the right side of the body, just posterior and lateral to the dorsal fin, was enucleated. It measured 8 × 10 cm in diameter, and weighed 550 g. An exploratory puncture yielded 45 ml of yellowish serous fluid.

In both instances, the tumour mass was sharply delimited by a fibrous capsule. There was no evidence of invasion of the adjacent tissues by tumour cells. No metastases or other tumours were found in either fish.

Incision of the tumour masses revealed a yellowish-white, fibrous tissue matrix with many cavities throughout the stroma (Fig. 2). Tissue samples were fixed in 10% formalin and processed by standard histological techniques. Tissue sections, 6 μ thick, were stained with haematoxylin and eosin and with a trichrome (haematoxylin–phloxine–safran) stain.

The stained sections revealed whorls and interlacing bundles of fibroblasts and collagen fibres (Fig. 3). The tumour cells were fusiform with large elongated nuclei and multiple nucleoli. Mitotic figures were not seen. On the basis of cellular morphology and staining response, both tumours were identified as fibromas.

The fluid aspirated from both tumours was examined for bacteria by culture and by Gram staining of centrifuged samples. No bacteria were detected.

Fibroma in species of trout has been reported only in brown trout (*Salmo trutta* Linnaeus (syn. *Salmo lacustris* L.)) in Switzerland by Eberth in 1878 (Schlumberger and Lucké 1948) and in Norway by Kreyberg (1937); and in a rainbow trout (*Salmo gairdneri* Richardson) in the USA by Nigrelli (1951). This is the first report of subcutaneous fibroma in the lake trout *Salvelinus namaycush* (Walbaum).

KREYBERG, L. 1937. An intra-abdominal fibroma in a brown trout. Amer. J. Cancer Res. 30: 112–114.

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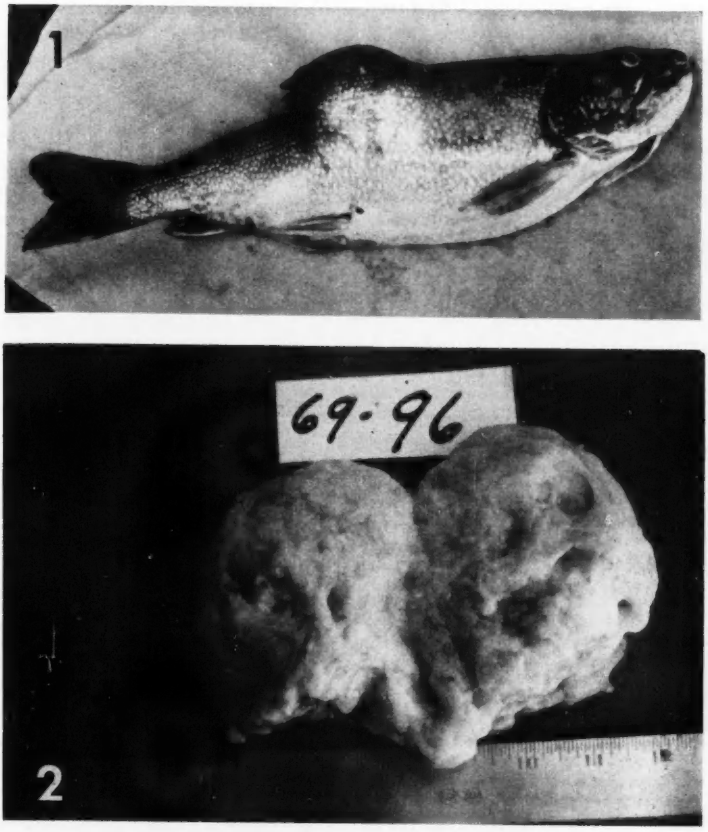

FIG. 1. Subcutaneous fibroma in the lake trout, *Salvelinus namaycush* (Walbaum).
FIG. 2. Appearance of incised tissue mass. Note the cavities in the fibrous stroma.

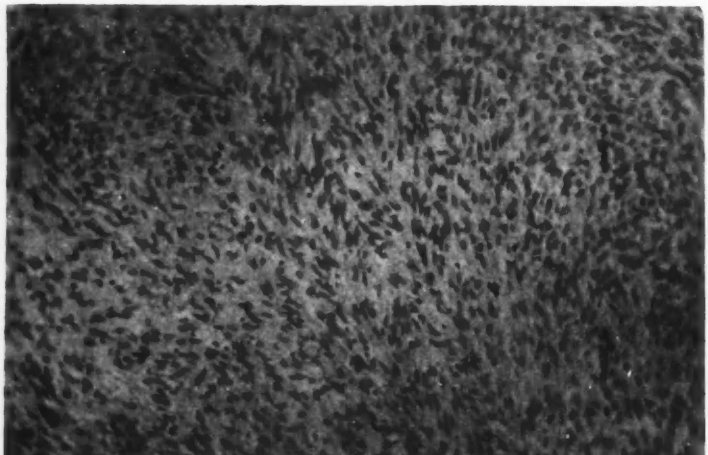

FIG. 3. Photomicrograph of tissue section showing whorls and interlacing bundles of fibroblasts and collagen fibres. ×150.

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Nitrogen Excretion and Protein Consumption of the Bluegill Sunfish (*Lepomis macrochirus*)

J. SAVITZ[1]

Department of Zoology
Indiana University, Bloomington, Ind.

SAVITZ, J. 1971. Nitrogen excretion and protein consumption of the bluegill sunfish (*Lepomis macrochirus*). J. Fish. Res. Bd. Canada 28: 449–451.

Nitrogen excretion rates increase with an increase in protein consumption levels. A mathematical description of this relation was formulated from the data. Estimates of the effect of specific dynamic action on nitrogen excretion were also calculated.

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THE purpose of this research was to study the relation between protein consumption levels in bluegill sunfish (*Lepomis macrochirus*) and their nitrogen excretion rates and to present a mathematical description between protein consumption and nitrogen excretion. Paloheimo and Dickie (1965, 1966) concluded that total metabolism increases with increasing ration levels and formulated an equation to describe this relation. Kausch (1969) also found an increase in oxygen consumption with feeding.

Methods — The methods of this investigation are very similar to those reported earlier (Savitz 1969a) and only differences in the present study will be explained in detail. The bluegills were kept in a large holding tank and fed mealworms (*Tenebrio molitor*). Before experiments, fish were anesthetized with 0.003% M.S. 222 and weighed. M.S. 222 does not appreciably affect the nitrogen excretion rates of bluegill (Savitz 1969b). One day after weighing, each fish was placed in a separate 5-gal aquarium that contained 8, 10, or 12 liters of aerated well water held at 23.9 C. Fish were not anesthetized while being handled.

The basic experimental procedure involves placing bluegills in a known quantity of water, feeding them a known amount of protein, and 1 day after feeding, measuring the amount of nitrogen excreted into the water. An important assumption is that protein intake equals protein digestion and absorption. Nearly 95% of the total protein fed was absorbed by longear sunfish (*Lepomis megalotis*) and green sunfish (*Lepomis cyanellus*) (Gerking 1952). When chitin nitrogen is not included, nearly 100% digestion occurs within 18 hr after bluegills are fed mealworms or natural food organisms (Windell 1966).

Fish were fed a constant number of mealworms each morning for 4 days. Beginning on the morning after the initial feeding, duplicate 25-ml samples of water were taken daily from each aquarium and analyzed by the micro-kjeldahl method. From these analyses, the nitrogen excreted in 1 day was calculated. After the 5th morning, fish could be placed in another aquarium with freshly aerated well water and the experiment continued.

Mealworms were chosen because previous studies (Gerking 1952, 1955a, and unpublished data; Windell, 1966) have shown that bluegills will ingest an entire mealworm at one time, thus ensuring against nutrient loss. Also, mealworms could easily be obtained in sufficient quantities. Samples of mealworms were analyzed by the micro-kjeldahl for nitrogen. LaFon (1942–44) found that 4.8% of the dry weight of mealworms was chitin, and chitin contains 6.9% nitrogen. The chitin nitrogen was calculated and subtracted from total nitrogen since chitin is not digested nor used for energy by fish. All other nitrogen was considered to be part of protein.

The seven fish in the experiment ranged in weight from 18.5 to 37.3 g, with an average weight of 28.9 g,

[1]Present address: Department of Biology, Loyola University of Chicago, 6525 North Sheridan Road, Chicago, Illinois 60626, USA.

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and food consumption ranged from 0.3 to 1.9% of this average. The data for all fish were combined in the results. Each fish was fed, at least, at two of three general feeding levels: high (11–15 mg N/day), medium (6–10 mg N/day), and low (2–4 mg N/day).

Precautions were taken to avoid erroneous nitrogen excretion rates. Samples that represent days when fish were handled were eliminated since handling may influence nitrogen excretion. When the size of meals was changed, there appeared to be a transition period of 1 day for nitrogen excretion rates to follow. Samples during these transition periods were also eliminated. Sources of error in this method have been discussed previously by Gerking (1955b) and Savitz (1969a, b).

Results — There is an increase in nitrogen excretion rate with an increase in nitrogen consumption level (Fig. 1). This result agrees with the conclusions reached by Paloheimo and Dickie (1966) and Kausch (1969). A difficulty arises in finding the proper mathematical description of the data. A possible equation describing this data is

$$T = aR^b \quad (1)$$

or in its linear form

$$\log T = \log a + b \log R, \quad (2)$$

where T is metabolic rate and R is ration level rate expressed as mg N/day. The a value is the y intercept, and the b value is the slope or ration level exponent. This relation between metabolic rate and ration level is shown in Fig. 1. These equations (1 and 2) are similar to those showing the relation between metabolism and body weight, and can be applied only to fish of similar body weights. The following equation was calculated from the data:

$$\log T = 0.07874 + 0.71119 \log R. \quad (3)$$

The 95% confidence limits for the slope are ±0.13926.

Discussion — Equation 3 adequately describes the data of this study, but the interpretation of the results could be limited by the relatively small range in ration levels; also, only seven fish of different sizes were used. There is a large variation in the results, part of which can be attributed to differences in body weight, but this variation is not unusual in metabolic studies of fish.

If equation 3 is used, a bluegill in this experiment would excrete 2.62 mg N/day at a feeding rate of 3.00 mg N/day, 5.72 mg N/day at 9.00 mg N/day, and 8.23 mg N/day at 15.00 mg N/day. Kausch (1969) and Warren and Davis (1967) credited specific dynamic action (SDA) for metabolic increases of feeding fish. Warren and Davis (1967), using Brocksen's (MS 1966) data, estimated SDA by comparing energy content of fish fed at different rates to starved fish. The energy content of starved fish decreased; the decrease was assumed to be equal to the energy used for activity in fed fish. That part of the calculated metabolic rate above that of starved fish was considered to be due to SDA. With these assumptions, if the nitrogen

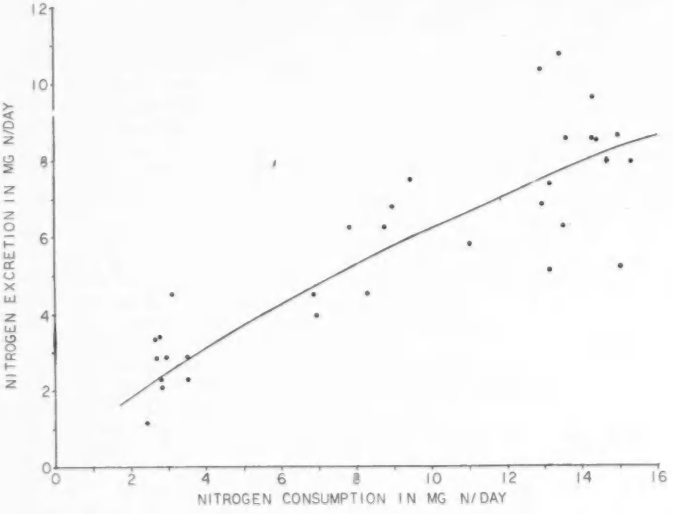

FIG. 1. Relation between nitrogen excretion rate and nitrogen consumption rate.

excretion rate of starved fish is subtracted from average nitrogen excretion rates of bluegills fed at different ration levels, the amount of nitrogen excreted for which SDA is responsible can be estimated. Three fish that were used in feeding experiments were starved; they reached a rather low constant nitrogen excretion rate on the 2nd and 3rd day of starvation. The average rate was 2.77 mg N/day, with a range from 1.27 to 4.09 mg N/day. The nitrogen excretion rate attributable to SDA is zero at a feeding rate of 3.00 mg N/day, 2.95 mg N/day at 9.00 mg N/day, and 5.46 mg N/day at 15.00 mg N/day.

Protein maintenance level is intimately connected to metabolism. Maintenance levels have been determined by either measuring nitrogen excretion or measuring protein content increases in animals with a known protein intake. In the latter type of study, the difference between protein intake and protein content increase is the amount of protein used for energy. Gerking (1955b) and Savitz (1969a) measured endogenous nitrogen excretion (ENE) of glucose-fed fish. This method of determining protein maintenance indicates the amount of tissue protein that has been used for energy and that must be replaced for the fish to maintain the same size. Savitz (1969a) compared ENE with protein maintenance levels of Gerking's (1952, 1955a) protein-fed fish and found that ENE provided a conservative measure of maintenance level.

From an equation from Savitz (1969a) showing the relation between body weight and ENE rates at 23.9 C, the ENE rate for a bluegill weighing 28.9 g (the average weight of fish in this experiment) is 3.55 mg N/day. This is higher than the rate of 2.62 mg N/day obtained at a low feeding rate of 3.00 mg N/day, but is lower than nitrogen excretion rates at feeding rates of 9.00 and 15.00 mg N/day. Apparently, at low ration levels, there is no appreciable difference between nitrogen excretion rates of glucose- and protein-fed fish. However, at higher ration levels, ENE rates are conservative. Maintenance level at higher rations depends on replacement of tissue protein and food protein used for energy through SDA.

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World Distribution of Brook Trout, *Salvelinus fontinalis*: Further Observations

HUGH R. MACCRIMMON, BARRA L. GOTS, AND J. SCOTT CAMPBELL[1]

Department of Zoology
University of Guelph, Guelph, Ont.

MACCRIMMON, H. R., B. L. GOTS, AND J. S. CAMPBELL. 1971. World distribution of brook trout, *Salvelinus fontinalis*: further observations. J. Fish. Res. Bd. Canada 28: 452–456.

This note supplements the original paper by MacCrimmon and Campbell (J. Fish. Res. Bd. Canada 26: 1699–1725, 1969) by presenting additional information on the native range, attempted introductions, and present world distribution of the brook trout.

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BY documenting further details on the pristine and present world distribution of the brook trout, *Salvelinus fontinalis* (Mitchell), this note supplements the previous account (MacCrimmon and Campbell 1969). Additions and modifications to the data published previously are the result of personal communications arising from the original paper and the use of several old publications not cited in the original paper.

Native range — It is questionable if the endemic range of the brook trout in Wisconsin was as general as previously described based on Brasch et al. (1966). In an earlier paper, Catlin (1930) defined the native distribution in Wisconsin to include only those streams on the west bank of Lake Michigan as far south as the Oconto River, a tributary of Green Bay. This reduced range places the natural southern distribution of the species in the Lake Michigan watershed at approximately the same latitude in Wisconsin as in Michigan. Otherwise, the limits of the native range of the brook trout would seem to be as previously described.

Introductions and Naturalized Distribution

NORTH AMERICA

The United States — A review of other early Government documents, particularly accounts by McDonald (1887), Worth (1893), Titcomb (1902), Henshall (1906), Bowers (1912), and O'Malley (1918), has shown minor differences with those dates of first introduction to the various states recorded in our earlier paper (Table 1). However, these data indicate no variance with the previously published early distribution of the species in the United States. Although we reported that attempts to sustain rainbow trout populations on a put-take basis in northern Ohio were discontinued in 1962 (Lakes, personal communication), Cold Creek (tributary to Sandusky Bay of Lake Erie) is now stocked annually with brook trout reared privately at the Castalia Farms (Robertson, personal communication).

Canada — The northern distribution of the brook trout has been extended to include lakes near Fort Smith in the Northwest Territories by recent and apparently successful plantings (Johnson, personal communication). Our earlier paper did not document the presence of a Provincial fish culture programme in the Province of Québec where, by 1966, domesticated hatchery brood stock had been replaced by a strain indigenous to the Laurentides Park for stocking fishing waters (Québec Department of Tourism, Fish and Game 1966).

Mexico — More specific details, following the proposal of Berriozábal (1937) that a brook trout hatchery be established, reveal that the first brook trout to be distributed from the El Zarco Fish Station, State of Mexico, were 1500 fish planted in the Río Tendido near Fortin, Veracruz in 1939 (Jara C. 1945). By 1945, some 91,838 brook trout had been distributed to waters in six states. However, the species is now present principally only in the states of Puebla and Mexico (Medina-Gandara, personal communication).

[1]Present address (J.S.C.): Department of Forestry and Natural Resources, University of Edinburgh, Edinburgh, Scotland.

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EUROPE

France — An earlier date than 1883 for the first importation of brook trout into France is given by Bean (1895) who stated that brook trout were shipped by the United States Fish Commission to the Trocadéro Aquarium of Paris in 1879 and subsequently introduced into the waters of France.

Sweden — A more complete account of the present status of the brook trout in Sweden has been provided by Nyman (personal communication). Self-sustaining populations occur in most Provinces and are the most abundant in Västergötland, Småland, Närke, Västmanland, Dalarna, Hälsingland Medlepad, Jamtland, Åugermanland, Västerbotten, Norrbotten, and Lappland. Waters in the woodlands of the latter three provinces seem best suited to the species. Introductions have been particularly successful in the Province of Norrbotten in an area where rainfall is less than 35 cm and considerably less than the 50 cm considered to generally delimit the European distribution of the species (Nyman, personal communication).

Fish culture provides trout that are planted only in waters below 600 m altitude, naturalized populations becoming established generally only below 200 m. In 1968, there were 25 fish farms rearing brook trout (Ljungberg, personal communication).

Yugoslavia — Brook trout are now known to be naturalized in some waters of Slovenia and near the source of the River Bosna at Sarajevo where they coexist with brown trout (Pažur, personal communication). The date of the introduction of brook trout (*potočna zlatovčica*) to Yugoslavia is unknown.

ASIA

Lebanon — An attempt by the Ministry of Agriculture to introduce brook trout from Denmark in 1960 proved unsuccessful (Hoyek, personal communication).

SOUTH AMERICA

Peru — Although it was noted previously that salmonid species were introduced to Peru first in 1928 (Tovar S., personal communication), Vildoso B. (personal communication) states that brook trout were first brought to the Chucuito Fish Culture Station in Puno from the United States

TABLE 1. Dates of first importations of brook trout in the United States of America.

State	Year of introduction	State	Year of introduction
Alabama	About 1902	Montana	1889
Alaska	1918	Nebraska	1882
Arizona	About 1912	Nevada	1880
Arkansas	1893	New Hampshire	Native
California	1872	New Jersey	Before 1884
Colorado	1874	New Mexico	1892
Connecticut	Native	New York	Native
Delaware	About 1912	North Carolina	About 1902
Florida	None	North Dakota	1902
Georgia	About 1912	Ohio	1868
Hawaii	1876	Oklahoma	None
Idaho	1902	Oregon	1893
Illinois	Late 1800's	Pennsylvania	1902
Indiana	Around 1902	Rhode Island	Native
Iowa	1895	South Carolina	Unknown
Kansas	None	South Dakota	1886
Kentucky	1918	Tennessee	Around 1902
Louisiana	None	Texas	None
Maine	Native	Utah	1875
Maryland	1887	Vermont	Native
Massachusetts	Native	Virginia	1887
Michigan	1879	Washington	1894
Minnesota	1878	West Virginia	1878
Mississippi	None	Wisconsin	About 1887
Missouri	1902	Wyoming	1880

in 1950 for stocking Lake Titicaca and its tributaries. The resulting naturalized population is described in our earlier paper.

AFRICA

South Africa — The only evidence of naturalized brook trout in the waters of South Africa is the presence of the species in mountain headwaters of a tributary of the Olifants River in the eastern Transvaal (Le Roux and Steyn 1968).

Rhodesia — Both brook trout and tiger trout (brown × brook hybrid) have been stocked in Rhodesian waters. Brook trout are now present in some waters of the Inyanga Mountains (Jubb 1961; Lingen, personal communication).

Kerquelen Islands — Brook trout were imported to these islands sometime prior to 1958 and have become naturalized in small lakes where spawning extends from March to May (Vibert, personal communication).

Discussion — An analysis of correspondence resulting from the previous publication on the world distribution of brook trout (MacCrimmon and Campbell 1969) confirms a general acceptance of the limits of native and naturalized range as therein described. Modifications in world distribution presented in this note (Fig. 1) include a slight reduction of the native range in Wisconsin on the United States side of the Great Lakes, an expansion of the nonindigenous distribution in the Northwest Territories of Canada, a more extensive naturalized distribution in Sweden, and the presence of naturalized populations in Yugoslavia and in the Kergelen Islands of the Indian Ocean.

The world distribution of the brook trout is presently more restricted in waters beyond its native range than either the brown trout (MacCrimmon and Marshall 1968; MacCrimmon et al. 1970) or rainbow trout (MacCrimmon and Gots unpublished data), although the species has become naturalized in all continents except Australia and Antarctica through introductions. Undoubtedly

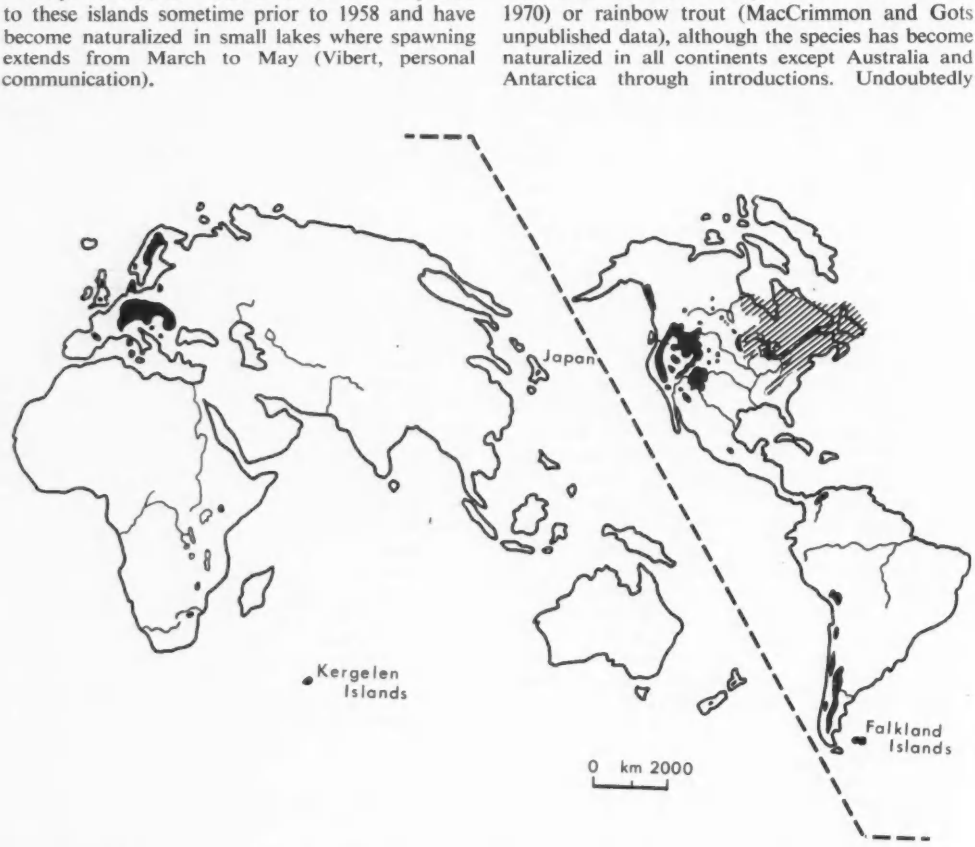

FIG. 1. Known world distribution of brook trout, *Salvelinus fontinalis*. Native range indicated by shaded area; naturalized by solid areas.

the relatively narrow ecological tolerance of the brook trout has been a factor in limiting both its natural and naturalized range. This environmental sensitivity is illustrated by the present absence of the species from the southern limits of its natural range in the States of Minnesota, Wisconsin, Illinois, Ohio, Pennsylvania, and New Jersey to the south of the Great Lakes where changing land- and water-use practices have caused the warming of summer water temperatures, increased siltation, and other forms of pollution. The species has persisted within the southern part of its native range only in the cool unsilted waters of mountainous areas reaching southward along the Appalachian Mountains to Georgia. Similarly, introductions resulting in the establishment of self-sustaining populations elsewhere have been limited essentially to cool clear waters of the world situated in temperate and subarctic regions and only in mountainous areas at lower latitudes in both hemispheres.

A second factor that has limited the world distribution of the brook trout is the often low public regard for the species beyond its native range. The reason for this lack of prestige when compared with locally indigenous salmonids would seem to lie in the common planting practice of placing brook trout in isolated headwater streams where cold water temperatures greatly inhibit available food and growth. Consequently, world culture and distribution of the brook trout has not been as extensive as for either the brown or rainbow trout.

As habitat conditions required by self-sustaining brook and brown trout populations are surprisingly alike (Marshall and MacCrimmon 1970), a comparison of present world distribution of the two species suggests that the range of the brook trout could be extended further through appropriate plantings and that, in environments similar to those within its native range, growth rates would approximate those occurring among indigenous landlocked and anadromous populations. However, further extension of the brook trout range must be based on the considered need for the introduction of the exotic species to local fisheries.

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Fecundity of the Yellowtail Flounder (*Limanda ferruginea*) from the Grand Bank, Newfoundland

T. K. PITT

Fisheries Research Board of Canada
Biological Station, St. John's, Nfld.

PITT, T. K. 1971. Fecundity of the yellowtail flounder (*Limanda ferruginea*) from the Grand Bank, Newfoundland. J. Fish. Res. Bd. Canada 28: 456–457.

Fecundity estimates made on 51 mature yellowtail flounder (*Limanda ferruginea*) from the Grand Bank ranged from 350,000 to 4,570,000 eggs per female. Fecundity depended mainly on body length and to a lesser extent on age.

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THIS report is an analysis of the fecundity of the yellowtail flounder (*Limanda ferruginea*) from the Grand Bank of Newfoundland on the basis of 51 ovaries. No information is available in the literature on the fecundity of this species. Appreciable quantities of this flatfish, averaging 6 million lb (2700 metric tons) annually, have been landed from the Grand Bank by Newfoundland trawlers since 1965, and it has been of considerable importance to the New England otter-trawler fishery for some time.

Material and methods — The ovaries for fecundity studies were collected during regular spring research cruises on the Grand Bank, 1966 and 1967; all were collected before the eggs became translucent. The ovaries were placed in Gilson's fluid (as modified by Simpson 1951) for about 3 months. Cleaning and counting of the eggs were as described by Pitt (1964) for American plaice except that frequently, because of the large number of eggs, smaller samples, i.e. 1/5000 or 1/2500 instead of the usual 1/1000, were used. The diameters ranged from 0.30 to 0.50 mm after preservation in Gilson's fluid and formalin.

Results and discussion — The usual method of relating fecundity to length and age by converting to logarithms was employed to give the following relations:

(1) Fecundity vs. length (Fig. 1A)

$$\log F = 4.69 \log L - 1.45$$
$$\text{or } F = 0.0355L^{4.69}.$$

The correlation coefficient 0.747 (Table 1) is significantly different from zero ($P < 0.01$).

(2) Fecundity vs. age (Fig. 1B)

$$\log F = 2.10 \log A + 4.31$$
$$\text{or } F = 20550A^{2.10}.$$

The correlation coefficient was 0.569 (Table 1), which is significantly different from zero ($P = 0.01$).

On the basis of these correlation coefficients it is apparent that fecundity is more dependent on length than on age. However, since length is a function of age, a multiple correlation was used to analyse the importance of each in relation to fecundity (Table 1). When length and age were combined, the coefficient of multiple determination was greater than when length alone was used. This increase in the amount of explained variation was significantly different from zero ($P = .03$) when the coefficient of partial determination ($r_{13.2}$) was used. Both length (55.8%) and age

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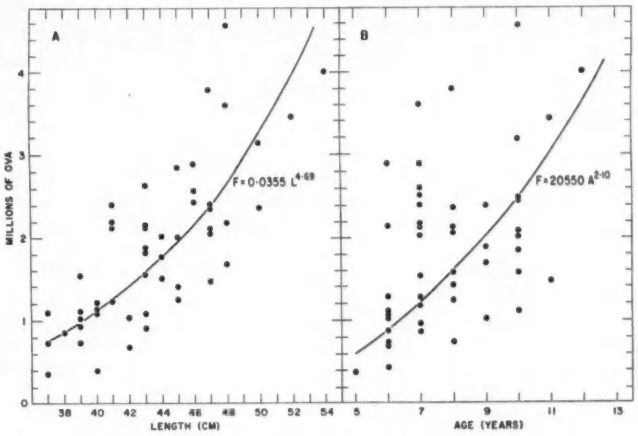

FIG. 1. Scatter diagram of yellowtail fecundity plotted (A) against length, and (B) against age.

(34.8%) contribute to the amount of variation observed. However, for all practical purposes one may conclude that fecundity is reasonably explained by the use of length alone.

The value of 4.69 found for the exponent in the length–fecundity relation is a little higher than that found for most fecundity relations. For other species of fish, the value has ranged between 3 and 4, tending toward the cube of the length as was found by Simpson (1951) for North Sea plaice (*Pleuronectes platessa*). However, values higher than 4 were found for Irish Sea herring, 4.5 (Farron 1938); Grand Bank haddock, 5.1 (Hodder 1963) and Greenland halibut, 4.66 (Lear 1970).

In the fecundity–age relation the increase in fecundity was approximately proportional to the square of the age and was similar to that reported for other Northwest Atlantic species: Grand Bank haddock, 2.0 (Hodder 1963); Atlantic cod, 1.95 (May 1967); and Greenland halibut, 1.95 (Lear 1970).

TABLE 1. Summary of statistics for multiple correlation of fecundity, age, and length. X_1 — log fecundity, X_2 — log length, and X_3 — log age ($X_1 - {}^a1.23 + {}^b12.3X_2 + {}^b13.2X_3$).

No. fish:	N	51
Coefficient of:		
determination for X_1 and X_2	r^2_{12}	0.558
correlation for X_1 and X_2	r_{12}	0.747
determination for X_1 and X_3	r^2_{13}	0.348
correlation for X_1 and X_3	r_{13}	0.569
multiple determination	$R^2_{1.23}$	0.616
multiple correlation	$R_{1.23}$	0.785
partial determination, X_1 and X_3	$r^2_{13.2}$	0.113
	t for $r_{13.2}$	2.344
	P for $r_{13.2}$	0.02–0.025

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Response of Brook Trout (*Salvelinus fontinalis*) Populations to a Fishery

A. L. JENSEN[1]

Environmental Biology Branch
Division of Environmental Research and Development
Tennessee Valley Authority, Muscle Shoals, Alabama 35660 USA

JENSEN, A. L. 1971. Response of brook trout (*Salvelinus fontinalis*) populations to a fishery. J. Fish. Res. Bd. Canada 28: 458–460.

From data in the literature, life tables are calculated for seven different brook trout (*Salvelinus fontinalis*) populations and used to study the mechanisms that reestablish the balance of births and deaths upset by fishing. It appears that a change in age-specific fecundity is the compensatory mechanism in brook trout.

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IF a fish population is not fished, reproduction is balanced by natural mortality. If fishing begins, the balance is tipped toward greater mortality, and fishing will reduce the usable stock until it becomes commercially or totally extinct or until a new balance is established. Factors that have been considered important in establishing this new balance are recruitment, growth rate, and natural mortality (Ricker 1958). These factors have, therefore, formed the basis for mathematical analysis of equilibrium yield (Gulland 1967).

Ecological life tables, a relatively new technique in ecology, have only recently been used to study fish populations. Life tables record a series of measurements that show population change throughout the life history of a species. The measurements are recorded in a tabular form and there is no need to worry whether they conform to some special law. One or two life tables may not be useful, but a sequence of tables for the same and different populations could increase our understanding of fish population dynamics.

From data in the literature, life tables for seven brook trout, *Salvelinus fontinalis*, populations have been calculated (Table 1). In Table 1 are recorded age, x; the fraction of the population at each age, $c(x)$; age-specific fecundity, the number of female eggs produced per female per year, $m(x)$; and age-specific annual mortality rate, $a(x)$. From a life table, change in population density can be calculated using the equation (Keyfitz 1968),

$$\frac{dN}{dt} = \left[\sum_{x=0}^{v} c(x)m(x) - \sum_{x=0}^{v} c(x)a(x) \right] N. \quad (1)$$

Provided the population has a stable age distribution, annual rate of increase can be calculated as

Annual rate of increase =

$$\exp \left[\sum_{x=0}^{v} c(x)m(x) - \sum_{x=0}^{v} c(x)a(x) \right], \quad (2)$$

where N is the population density and v is the oldest age the fish attain. Data is available for calculating the annual rate of increase of four trout populations; except for the unexploited population studied by Saunders and Power (1970), the rates of change are nearly all zero. Saunders and Power (1970) have, as they suggest, underestimated the number of young, or else there were large fluctuations in year-class strength.

Six of the populations are from streams, whereas the one studied by Saunders and Power (1970) is from Lake Matamek, Quebec, and its tributary streams. In the Lake Matamek trout population there was a large number of older fish, and although mortality of both young and old was high, mortality of ages 1+ and 2+ was low (Table 1). Age-specific fecundity rates and growth rates were also low. The trout population in Lawrence Creek, Wisconsin, studied by McFadden (1961), was heavily fished. Few fish in this heavily exploited population lived more than 2 years; age-specific fecundity rates were high, growth rate was high, and age-specific mortality rates were high for all age-groups. The

[1]Present address: Division of Environmental Research and Development, Tennessee Valley Authority, Chattanooga, Tenn.

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population in Hunt Creek, Michigan, studied by McFadden et al. (1967), was fished but not as heavily as the Lawrence Creek trout population. A few fish lived more than 3 years, and age-specific fecundity was lower than in Lawrence Creek. Mortality of age 1+ trout was lower in Hunt Creek than in Lawrence Creek.

The trout populations studied by Wydoski and Cooper (1966) in Lingle Stream and Tomlit Run were lightly fished. In these populations a few fish lived more than 4 years. Mortality rates of young and old were high, but mortality rates of ages 1+, 2+, and 3+ were lower than the mortality rates in more heavily fished populations. Age-specific fecundity rates and growth rates in these populations were low. The trout populations in Larry Creek and Big Spring, two Pennsylvania streams studied by Cooper (1967), were unfished or only lightly fished. Fecundity data reported by McFadden et al. (1967) were used together with Cooper's data to construct life tables for these two populations. A few fish lived more than 4

TABLE 1. Life tables for brook trout populations subjected to different intensities of fishing (Saunders and Power 1970; McFadden 1961; McFadden et al. 1967; Wydoski and Cooper 1966; Cooper 1967). E = annual rate of increase; numbers in parentheses were calculated from fecundity and population density.

Age	$c(x)$	$m(x)$	$a(x)$	$c(x)$	$m(x)$	$a(x)$
	Matamek Lake[a]			Lawrence Creek[b]		
0+	0.9379	0	0.9757	(0.9871)	0	0.9892
1+	0.0228	0	0.0495	0.0106	71	0.8031
2+	0.0216	27	0.3483	0.0021	102	0.9195
3+	0.0141	82	0.7786	0.0002	84	0.9583
4+	0.0031	146	0.9058	0.0000	–	–
5+	0.0002	184	0.8533	0.0000	–	–
		E = 1.2881			E = –0.0034	
	Hunt Creek[c]			Lingle Stream		
0+	0.9444	0.0	0.9603	(0.9137)	0.0	0.9554
1+	0.0375	0.0	0.5947	0.0408	0.0	0.4110
2+	0.0152	38.4	0.8274	0.0240	3.3	0.3094
3+	0.0026	129.5	0.8972	0.0166	28.5	0.7083
4+	0.0003	303.1	1.0000	0.0048	74.7	1.0000
5+	0.0000	–	–	0.0000	–	–
		E = 0.0670			E = –0.0028	
	Tomlit Run[d]			Larry Creek[a]		
0+	(0.8232)	0.0	0.9057	(0.9860)	–	0.9934
1+	0.0776	0.0	0.0870	0.0066	–	0.5634
2+	0.0709	1.3	0.6799	0.0029	–	0.0000
3+	0.0227	20.0	0.7521	0.0031	–	0.5606
4+	0.0056	49.3	1.0000	0.0013	–	0.8966
5+	0.0000	–	–	0.0001	–	1.0000
		E = –0.0010				
	Big Spring[a]					
0+	(0.9677)	–	0.9805			
1+	0.0188	–	0.4726			
2+	0.0099	–	0.7016			
3+	0.0030	–	0.8649			
4+	0.0004	–	0.6000			
5+	0.0002	–	1.0000			

[a]Unfished.
[b]Heavily fished.
[c]Fished.
[d]Lightly fished.

years. Mortality of both old and young fish was high, but again mortality rates of ages 1+, 2+, and 3+ were lower than in more heavily fished populations.

The contrast between fished and unfished or lightly fished populations is sharp. A fact most clearly illustrated by the data is that although age structure and vital statistics differ considerably among the populations, reproduction always balances mortality. These tables reveal that the balance between reproduction and mortality is reestablished when a brook trout population is fished. Fishing clearly changes age structure, resulting in more young and fewer old fish. Increased fishing does not appear to affect mortality of the zero age-group. It is usually assumed that the main compensation to fishing occurs sometime during the first year of life. Beverton (1962) concludes that compensation in the adult stage and compensation in the reproductive process of plaice are minor as compared with compensation during early life, but no firm conclusions could be reached on the basis of available data.

The life tables suggest that fishing mortality is compensated by a higher age-specific fecundity. In unexploited populations with lower age-specific mortality rates for the middle age-groups, age-specific fecundity is lower than in exploited populations. Beverton (1962) concluded age-specific fecundity of plaice was reduced 15–20% when fishing stopped during World War II. The decrease in plaice fecundity was related to a smaller body size. McFadden and Cooper (1964) and McFadden et al. (1965) studied fecundity of brown trout in 11 different streams and concluded that in waters of low primary productivity few eggs are produced per adult, and that this lower rate of reproduction is important for keeping the trout population in equilibrium with its environment.

Apparently age-specific fecundity is relatively flexible. The brook trout populations even matured at an earlier age under heavy exploitation. Saunders and Power (1970) showed that lower age-specific fecundity of the trout population in Lake Matamek was related to a lower growth rate, and fecundity has been shown to increase with size (Bagenal 1967). Thus growth may indirectly be the most important variable in adjustment of a fish population to a fishery, as hypothesized by Nikolskii (1969). It is not known how generally this conclusion applies, but life tables could be useful for investigating this problem in other populations.

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A Record of the Atlantic Menhaden (*Brevoortia tyrannus*) from Northumberland Strait

W. H. LEAR AND J. H. C. PIPPY

Fisheries Research Board of Canada
Biological Station, St. John's, Nfld.

LEAR, W. H., AND J. H. C. PIPPY. 1971. A record of the Atlantic menhaden (*Brevoortia tyrannus*) from Northumberland Strait. J. Fish. Res. Bd. Canada 28: 461–462.

A single specimen of the Atlantic menhaden (*Brevoortia tyrannus* (Latrobe 1802)) was taken off South Kouchibouguac Bay in Northumberland Strait. Previous most northerly published records for the North Atlantic were from western Bay of Fundy and the most easterly record was from St. Mary's Bay, Nova Scotia.

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A specimen of the Atlantic menhaden (*Brevoortia tyrannus* (Latrobe 1802)) was taken in a surface trapnet set 3 miles from the shore in South Kouchibouguac Bay (46°50′N, 64°53′W) on June 16, 1970. The specimen was presented to the authors for identification.

Following are morphometric and meristic data for the specimen: greatest total length 333 mm; fork length 286 mm; greatest girth 202 mm; greatest body depth 86 mm; head length 83 mm; distance from snout to anterior insertion of dorsal fin 129 mm; minimum caudal peduncle depth 23 mm; eye diameter 15 mm; dorsal fin rays 19; anal fin rays 20; pectoral fin rays 17 (right), 17 (left); pelvic fin rays 7 (right), 7 (left); number of vertebrae 47 (by X-ray). These morphometric and meristic data are within the typical range reported by Jordan and Evermann (1896) and Leim and Scott (1966) for this species. The specimen was a female with developing ova apparent. The weight of the one whole gonad was 22.9 g. The average diameter of a random sample of 58 ova was 0.63 mm (range 0.23–1.36 mm; standard deviation 0.23 mm). The whole weight of the specimen was 347.9 g and the gutted weight was 297.7 g. The whole weight and possibly the girth may be underestimated since the fish was cut and part of one of the gonads was lost. The stomach was empty.

This specimen has extended the northerly range limit 104 miles or approximately 740 sea miles northward of the Bay of Fundy specimens reported by Perley (1852) and Leim and Day (1959). Also, it is 63 miles east of the previous easterly limit (Bigelow and Schroeder 1953), St. Mary's Bay, on the west coast of Nova Scotia. It is also north of the Nova Scotia range reported by Hildebrand (1948), although the specific area of Nova Scotia where the specimens were taken is not mentioned. It is highly probable, however, that the area referred to is southern Nova Scotia and Bay of Fundy.

Messrs K. T. MacKay and J. Gilhen of the Fisheries Research Board of Canada Marine Ecology Laboratory, Dartmouth, N.S., inform us that during 1970 there was an unusual northward movement of Atlantic menhaden to the Halifax area. The presence of these large schools of menhaden in this area may partly account for the occurrence of a stray specimen as far north as Kouchibouguac Bay.

There are two main routes by which our specimen could have reached the Northumberland Strait. The shorter route would involve swimming against the current for roughly 700 miles from the Gulf of Maine along the coast of Nova Scotia into the Gulf of St. Lawrence, either around Cape Breton Island or through the Strait of Canso. When we consider the current velocity along eastern Nova Scotia (MacGregor 1956) this route seems doubtful. Although the general circulation pattern along the coastal Nova Scotia Shelf is in a southwest direction (Trites and Banks 1958), there are fluctuations in the current directions, especially farther offshore where the current flows in a northeasterly direction (Hachey 1947). The fish may have been transported by one or more of these northeasterly eddies northeastward along the Nova Scotia Shelf. It could have swum through the Strait of Canso (under the causeway) or northeastward to Cape Breton Island from where it wandered into the Gulf of St. Lawrence to Kouchibouguac Bay.

This specimen is in the collection of the National Museums of Canada (Cat. No. NMC 70-230).

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Recent Articles on FRB Investigations Published in Other Periodicals

Until 1970, reprints of these articles had been included in an annual volume of "Studies," composed of two parts. The final binding was the 1969 Studies, Part 2, which included No. 1355. These are no longer bound and distributed. We continue, however, to number and list all articles, which henceforth may be obtained in reprint form only from the author or originating FRB establishment. The number following each title refers to the FRB establishment listed below.

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(7) Fisheries Research Board of Canada
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Halifax, Nova Scotia

(8) Fisheries Research Board of Canada
Biological Station
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